CIL

Cambridge Isotope Laboratories, Inc.

BUCHEM BV

www.isotope.nl

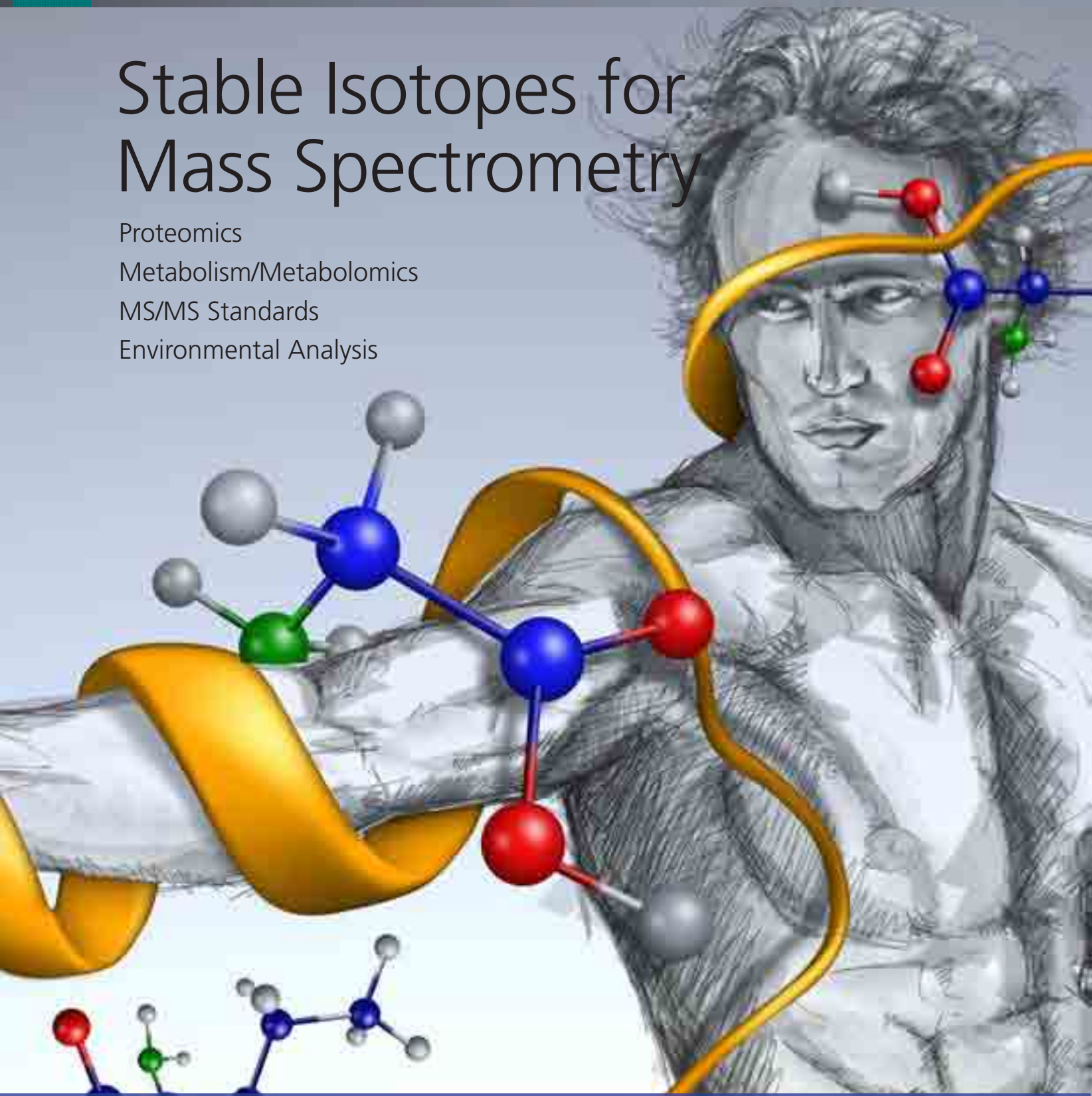
Stable Isotopes for Mass Spectrometry

Proteomics

Metabolism/Metabolomics

MS/MS Standards

Environmental Analysis



Orders BENELUX

Buchem B.V.
Minden 60/62
7327 AW Apeldoorn
The Netherlands

Tel +31 (0)55 543 00 40
Fax +31 (0)55 543 00 50
Email info@buchem.com
Web www.buchem.com
www.isotope.nl

Enriching Scientific Discovery

Welcome

Over the past decade, there have been vast improvements in the detection and quantification of proteins, metabolites and potential biomarkers using mass spectrometry-based methodologies. Advances in bioinformatics and instrumentation, combined with the use of stable isotopes, have furthered the development, sensitivity and accuracy of quantitative methods.

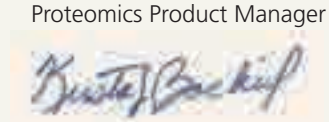
It is with great pride that we present Cambridge Isotope Laboratories, Inc.'s (CIL) new "Stable Isotopes for Mass Spectrometry" catalog. In this catalog, you will find a comprehensive listing of our isotope-enriched products that can be utilized for a wide range of mass spectrometry-based fields of research, including proteomics, metabolism, metabolomics, clinical diagnostics and environmental analysis.

Complementing the extensive product listing are contributions written from some of the world's most prominent mass spectrometrists, biochemists and research scientists. We are delighted to showcase application notes, customer perspectives and testimonials from many leaders in the field, illustrating the utility of stable isotopes. We would like to thank and show our appreciation to the many researchers who have contributed to this catalog. Please see page 163 for a complete list of contributors.

CIL continues to maintain a leadership role in developing new products to study proteins, protein turnover, metabolic disorders and environmental contaminants. It has been through our partnerships and close relationships with our customers over the past 30 years that we have been able to significantly expand our product offering in order to assist this community in the advancement of their studies utilizing stable isotope-labeled compounds as a tool in mass spectrometry. We welcome suggestions from our customers for new products, which will advance their research.

Our business is stable isotopes, but our focus is you, the customer. Thank you for giving us the opportunity to partner with you since 1981. You have truly made our success possible.

Tasha Agreste
Proteomics Product Manager



Krista Backiel
Metabolic Product Manager

Carol Beland
MS/MS Standards Product Manager

Terry Grim
Environmental Product Manager

Tasha Agreste, Carol Beland, Terry Grim and Krista Backiel

Cover illustration by Pedro Lalli

Cambridge Isotope Laboratories, Inc. Facilities

Cambridge Isotope Laboratories, Inc. (CIL) has state-of-the-art production facilities for cGMP and non-cGMP manufacturing at its locations in Andover and Tewksbury, Massachusetts.

CIL WORLD HEADQUARTERS AND cGMP PRODUCTION LABORATORIES TEWKSBURY, MA USA

Cambridge Isotope Laboratories, Inc. moved into its new Tewksbury, Massachusetts facility in the spring of 2013. As our new corporate headquarters, this 40,000-square-foot facility houses our executive team as well as our sales, marketing, finance, regulatory affairs and cGMP production staff. In addition to corporate office space, the facility has a 10,000-square-foot state-of-the-art cGMP suite which includes production laboratories, dedicated isolation rooms, a dedicated analytical laboratory, a packaging laboratory and a development laboratory.

CIL PRODUCTION LABORATORIES ANDOVER, MA USA

CIL's primary production facility in Andover, Massachusetts is dedicated to the manufacture of deuterated NMR solvents, stable isotope-labeled chemicals and gases, as well as specific cGMP products. This 57,000-square-foot facility is home to our operations staff and our production and quality-control teams.

The formulations group has years of experience formulating highly purified labeled materials into high quality quantitative solutions as analytical standards, either as single-component products or multi-component mixes and calibration solutions.

The quality-control lab is equipped with a wide array of instrumentation, including gas chromatograph/mass spectrometers (GC/MS), high field NMRs, HPLCs and an FT-IR. CIL's chemistry laboratories are equipped with apparatus for both large scale (50+ liters) and microscale chemistry, including equipment for high pressure gas reactions, pH and temperature-controlled enzyme chemistry, high resolution distillation processes and catalytic reduction with both hydrogen and deuterium. The production laboratories are also equipped with analytical equipment for in-process testing, including GC-FID, GC-ECD and HPLC with UV, RI, ELSD and MS detectors. All of these resources allow CIL to consistently produce products with high chemical and isotopic purity.

Production Spotlight

William Wood, PhD
Director of Chemistry

CIL has state-of-the-art production facilities for cGMP and non-cGMP manufacturing at its locations in Andover and Tewksbury, Massachusetts. The company employs over 40 chemists in production, more than half of whom hold higher degrees, with extensive years of experience exclusively in the synthesis of stable isotope-labeled compounds.

The production department has extensive experience in taking highly purified labeled materials and preparing high quality quantitative solutions as analytical standards, either as single-component products or multi-component mixes and calibration solutions. Using procedures that have been carefully developed and refined incorporating many years of experience in the field, the formulated analytical standards meet the most exacting requirements. The department has also prepared analytical standards and calibrators in reconstitutable dried-down formats for over 15 years. Where appropriate, these products are prepared under ISO 13485 or ISO 17025/Guide 34 quality systems.

CIL's chemistry laboratories are equipped with apparatus ranging from large scale (50+ liters) to micro-scale chemistry, including equipment for high pressure gas reactions, pH and temperature-controlled enzyme chemistry, high resolution distillation processes and catalytic reduction with both hydrogen and deuterium. The production laboratories are also equipped with analytical equipment for in-process testing, including GC-FID, GC-ECD and HPLC with UV, RI, ELSD and MS detectors. Automated separation equipment for preparative scale chromatography on silica gel and resin is also available, as is preparative GC.

Quality Assurance Spotlight

Ellen Veenstra
Quality Assurance/Regulatory Affairs Manager

CIL's independent Quality Assurance (QA) Department has a highly trained staff that is responsible for developing, maintaining, managing and improving the cGMP/ISO Quality Management System (QMS) to ensure that it is functioning in a compliant, efficient and effective manner. The QA department enforces that the high quality standard is adhered to within the company through effective training, internal audits and cross functional communication. The QA department is involved in all quality-related matters and must review and approve all appropriate quality-related documents. The main responsibilities of the QA department include, but are not necessarily limited to:

- Disposition of all cGMP/ISO Active Pharmaceutical Ingredients, products and/or intermediates, raw materials, *etc.*
- Ensuring deviations, out of specifications, complaints, and non-conformities are investigated and resolved.
- Providing on-going regulatory cGMP training.
- Internal and External Auditing

The QA department is also responsible for providing updates to executive management to keep them informed of any outstanding quality issues. Reports to management foster communication, review and refinement of QA activities to ensure that the Quality Program is adequate to meet or exceed regulatory and CIL's quality objectives.

Quality Control Spotlight

Tim Eckersley, PhD
Director of Quality Control

The CIL Quality Control (QC) laboratory staff specialize in the analysis and characterization of stable isotope-labeled compounds. Their expertise in this area makes the laboratory a world leader in this field. The majority of the staff has been with CIL for ten years or more.

There is a comprehensive quality system in place for analysis of both non-regulated and regulated materials. The quality system covers all aspects of testing, including training of personnel, control of documents, compliance with regulatory requirements, maintenance of equipment, generation of analytical records, general test methods, recording of test results and handling of out-of-specification results and materials.

The laboratory is audited on a regular basis by the FDA, ISO (to ISO 13485, ISO 17025 and Guide 34), by our customers and by our Quality Assurance department. In 2013 we expanded our quality system for environmental products to include ISO 17025 and Guide 34.

The laboratory handles testing for all of CIL's products and incoming raw materials, as well as in-process work for the production laboratories, shelf-life and stability studies. The materials range in complexity and physical form, from simple gases (*e.g.* labeled oxygen) to complex molecules like the macrocycle erythromycin. The laboratory is equipped to test and characterize the 15,000 different materials that constitute the CIL inventory and associated intermediates. Tests range in complexity from simple physical and spectroscopic characterization to chromatographic tests for purity, chirality and mass spectrometric testing for isotopic enrichment.

The in-house testing capabilities cover GC/MS, GC/FID, GC/ECD, HPLC/UV, HPLC/RI, HPLC/ELSD, HPLC/DA, HPLC/Pickering, ^{1}H-NMR, ^{13}C-NMR, Multi-nuclear NMR, Wet Chem, FTIR, TOC, Polarimetry, KF Testing. If the instrumentation required for a test is not available in-house, the testing is subcontracted to a qualified vendor.

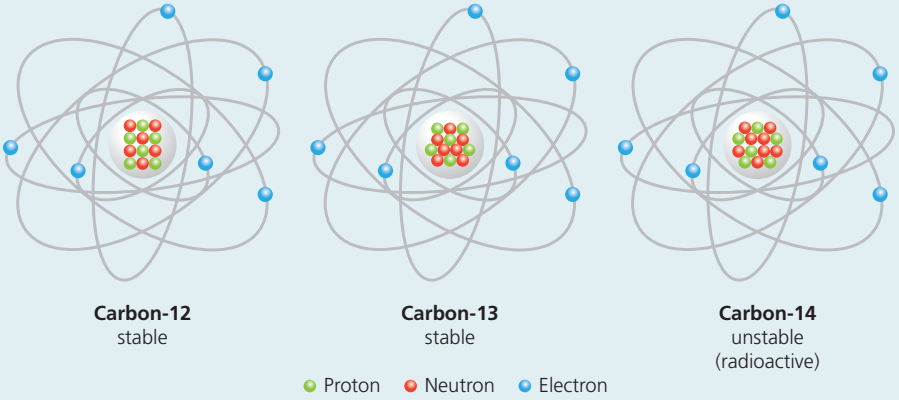
The laboratory has the personnel and systems in place to develop and validate new analytical methods, as well as to conduct testing according to all major standards. We regularly use USP/NF and EP compendia methods. The other compendia (BP and JP) are used as required.

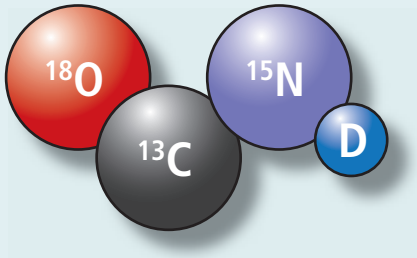
Table of Contents

Table of Contents

What Is an Isotope?

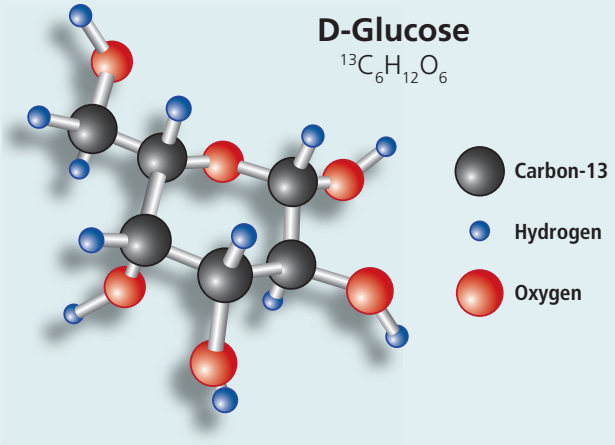
An isotope is any of two or more forms of a chemical element, having the same number of protons in the nucleus, or the same atomic number, but having different numbers of neutrons in the nucleus, or different atomic weights. There are 275 isotopes of the 81 stable elements, in addition to over 800 radioactive isotopes, and every element has known isotopic forms. Isotopes of a single element possess almost identical properties.





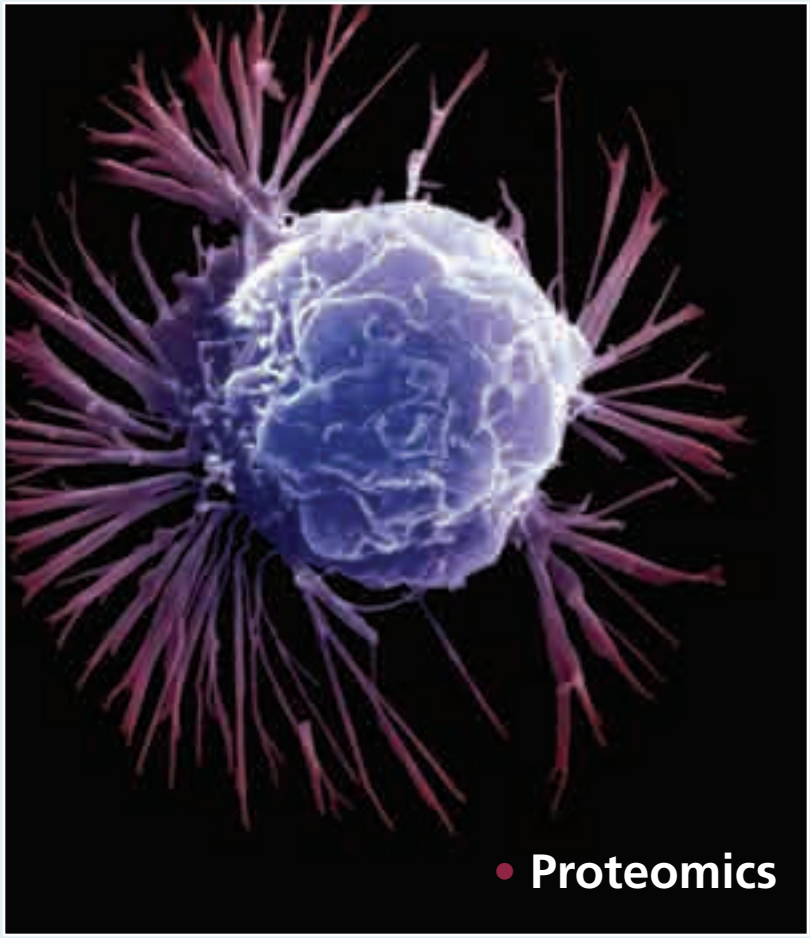
Calculating Isotopic Enrichment

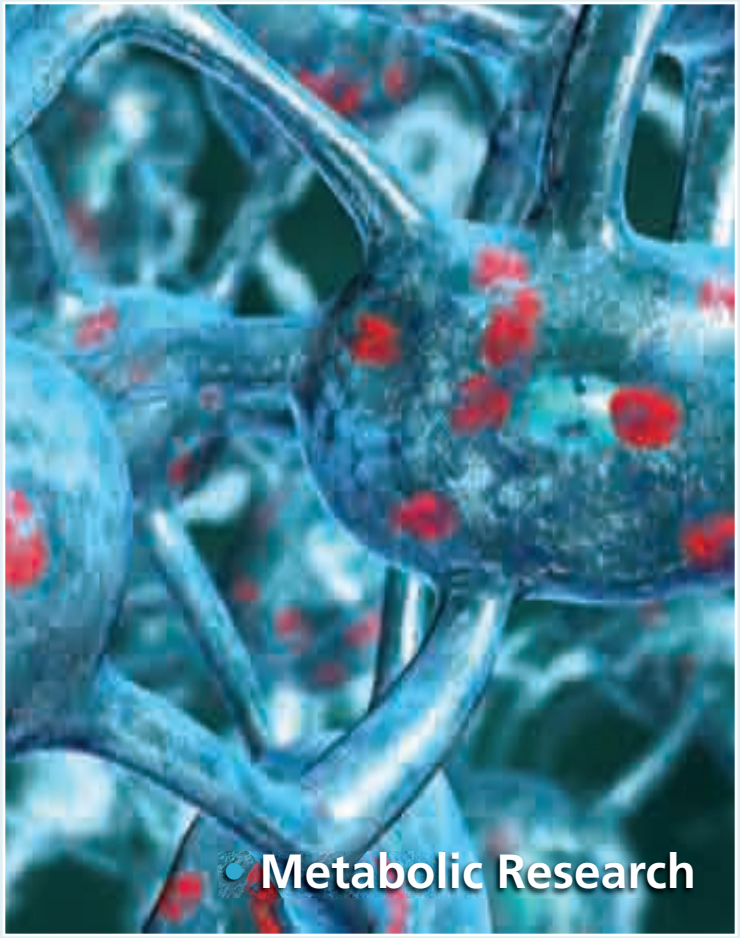
Isotopic enrichment is the average enrichment for each labeled atom in the molecule. **It is not the percentage of the molecules that are completely isotope labeled.** For instance, D-Glucose ($^{13}C_6$, 99%) is not 99% $^{13}C_6$, and 1% $^{12}C_6$. Each carbon atom position (1,2,3,4,5 and 6) has a 99% chance of being ^{13}C labeled and a 1% chance of being ^{12}C labeled. Thus, $(99\%)^6$ or ~94% of the molecules will have a molecular mass 6 AMU higher than native glucose and ~6% will have a molecular mass 5 AMU higher than native glucose. Theoretically, only $(1\%)^6$ or ~10^{-10}% will have the molecular mass of $^{12}C_6$ D-Glucose.

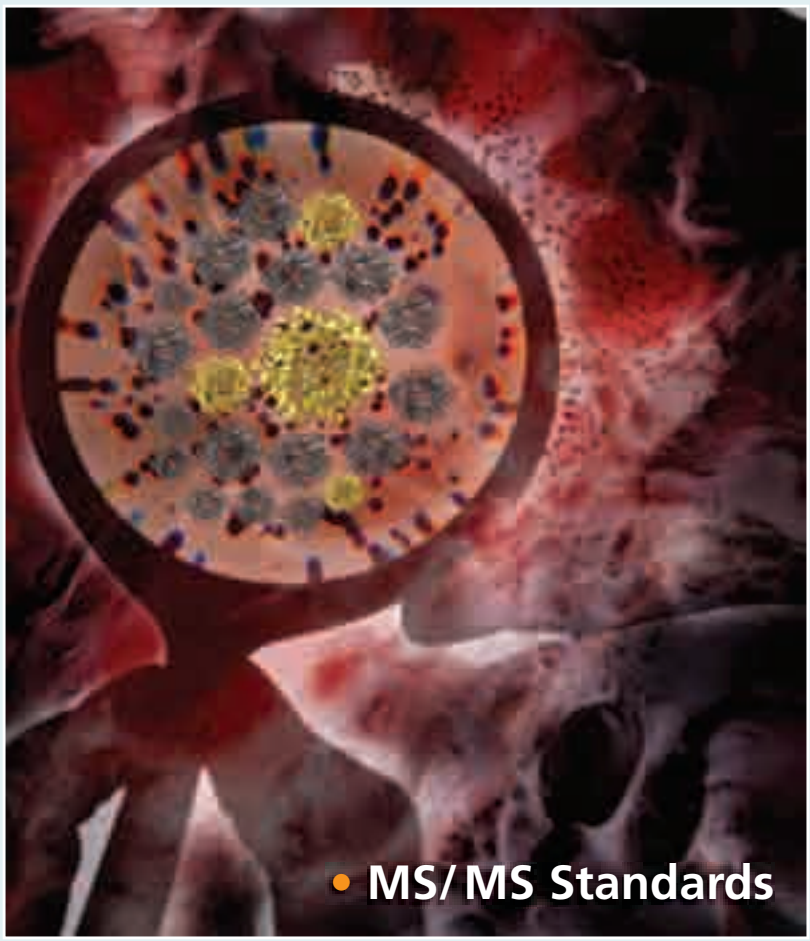


99% enriched

Please note: Products with high isotopic enrichment are denoted as **-H** and shaded gray throughout the catalog.


• Proteomics


• Metabolic Research


• MS/MS Standards


• Environmental

Utility of Stable Isotopes in Mass Spectrometry

Dwight E. Matthews, PhD
Professor of Chemistry and Medicine
The University of Vermont, Burlington, VT 05405 USA

The use of stable isotopes to define metabolic pathways and turnover of body constituents occurred very quickly after the discovery of deuterium and a method for isolating it, both by Harold Urey. Urey was awarded the Nobel Prize in 1934 for discovery of deuterium, but by the mid-1930s Rudolf Schoenheimer had already begun synthesizing deuterated molecules that he administered to rodents. With his young student, David Rittenberg, Schoenheimer defined synthesis and degradation pathways of many compounds, including fatty acids and cholesterol, that we take for granted today. When enriched nitrogen-15 (^{15}N) became available, Schoenheimer and Rittenberg demonstrated that proteins were dynamic in that they were both continually being synthesized and degraded.[1] All of this work was performed in only a few years using crude methods of preparation of labeled compounds and tedious measurement by isotope ratio mass spectrometry (IRMS) that requires all compounds be reduced to simple gases (CO_2, H_2, N_2) for measurement of isotopic enrichments.

After World War II, use of stable isotopes in biochemistry was mostly displaced by the availability of tritium and carbon-14 (^{14}C) radioisotopes. Although use of ^{15}N continued to study the nitrogen side of amino acid and protein metabolism and turnover (as there is no long-lived radioisotope of nitrogen), even this work was limited. It was not until the late 1960s that attention began to be paid to measurement of protein turnover using Glycine (^{15}N, 98%) (NLM-202) as the tracer and measurement of ^{15}N in urea, the end product of protein metabolism by Sir John Waterlow and colleagues.[2] There was a flurry of work in the 1970s, but again the primary reasons for the work were that Glycine (^{15}N, 98%) was easy to synthesize (the only amino acid without an optically active center), and the Urea ($^{15}N_2$, 98%+) (NLM-233) end product was easy to isolate from urine and prepare for measurement by IRMS. Use of ^{15}N and measurement directly in proteins was done, but that work was dwarfed by use of ^{14}C to make the same measurements.[2] Although both enriched carbon-13 (^{13}C) and deuterium were both available in the 1960s, their use as tracers in metabolism were extremely limited compared to ^{14}C and tritium.

The first major turning point for use of stable isotopes (^{2}H, ^{13}C, and ^{15}N) came with the development of gas chromatography/mass spectrometry (GC/MS). GC/MS provided separation of complicated mixtures of components and the ability to measure mass differences in those compounds. However, early GC/MS instruments were magnetic sector mass spectrometers. Nonetheless, Sweeley developed a system to perform limited selected ion monitoring (SIM) on an early instrument and demonstrated measurement of D-Glucose (1,2,3,4,5,6,6-D_7, 98%) (DLM-2062).[3] As quadrupole GC/MS instruments arose, measurement of stable isotopically labeled enrichments became common place over a wide range of compounds.[4] Stable isotopically labeled compounds (often deuterated) were used as internal standards for quantification and were proposed as "gold standard" methods for clinical chemistry measurement of even simple compounds, such as glucose in plasma. The other more important use of GC/MS was for measurement of stable isotopically labeled compounds as *in vivo* tracers for studies of metabolite kinetics in mammals.[4]

Two notable tracers arose in the 1970s that have become standard tracers reported in hundreds of studies and administered to thousands of people: D-Glucose (6,6-D_2, 99%) (DLM-349) for measuring the rate of glucose production and L-Leucine (1-^{13}C, 99%) (CLM-468) for measuring the rate of protein turnover and oxidation.[4] These compounds became popular only because companies, such as CIL, were able to develop cost-effective syntheses of the optically active compounds in large quantities. Because a gram of D-Glucose (6,6-D_2, 99%) may be administered intravenously, orders for the material are often 100 grams or more. Use of both of these compounds relied on GC/MS, but GC/MS is limited in terms of how low an enrichment can be measured. The development of GC-combustion-MS (GC-C-MS), also in the 1970s,[5] added a method for measuring very low enrichments of stable isotopically labeled tracers. This technique allowed relatively straightforward direct measurement of protein synthetic rates and could be defined as a very early proteomic method.

GC/MS remained the method of choice for the greatest range of stable isotope tracer measurements until the invention of electrospray ionization (ESI) by Fenn in the 1980s and its commercialization as the interface for liquid chromatography/mass spectrometry (LC/MS) in the 1990s. ESI-LC/MS allowed measurement of much larger and much more polar molecules, such as peptides, that could never be measured by GC/MS. ESI-LC/MS along with MALDI-TOF opened the door for mass spectrometry into proteomics. Just as GC/MS was initially used primarily to identify compounds, so was LC/MS initially used in proteomics to identify peptides. However, the next logical step was quantification with identification, and we again return to

the use of stable isotopically labeled compounds as internal standards.

The simplest and most cost-effective isotopically labeled internal standards were not labeled peptides, but labeled derivatization reagents. Peptides derived from one protein sample were chemically modified with an unlabeled derivatization reagent and combined with peptides from another protein sample that were chemically derivatized with a stable isotopically labeled reagent, and the ratio of the unlabeled-to-labeled peptides measured.[6]

For cell culture experiments, cells could be directly grown using stable isotopically labeled substrates to incorporate stable isotope labels directly into cellular proteins.[7] For simple cells such as yeast,[8] the media could be D-Glucose (^{13}C) and/or ammonium salts (^{15}N). For mammalian cells, the labels had to be introduced directly as labeled amino acids, typically labeled lysine and arginine. Stable isotope companies, such as CIL, developed products to make growing labeled cells in culture no more difficult than growing normal cells. However, the ultimate stable isotopically labeled internal standards in proteomics has been the growth of whole labeled animals, initially a ^{15}N-labeled rat,[9] but later as a range of small animals from mice[10] to worms.[11] Labeled protein, even as prepared animal feed, can be purchased directly from companies, such as CIL, for growing your own stable isotope-labeled animal to encompass all stable isotope possibilities.

The whole process of use of stable isotopes has evolved around both technology development for innovative mass spectrometric techniques to measure molecules in complicated matrices and technology development of cost-effective preparation of stable isotopes at high isotopic enrichments and strategies for incorporation of the isotopes into organic molecules. It is interesting to note that initially all incorporation was done via classical organic chemical synthesis, but more recently methods have been developed to incorporate isotopes into molecules using biochemical and cell biological techniques. One point is true: as biological science evolves, use of stable isotopes will evolve with it and remain a key tool in research.

References

1. Schoenheimer, R. **1942**. The Dynamic State of Body Constituents. Cambridge, MA: *Harvard University Press,* 1-78.
2. Waterlow. J.C.; Garlick, P.J.; Millward, D.J. **1978**. Protein Turnover in Mammalian Tissues and in the Whole Body. Amsterdam, North-Holland, 1-804.
3. Sweeley C.C.; Elliott, W.H.; Fries, I.; Ryhage, R. **1966**. Mass spectrometric determination of unresolved components in gas chromatographic effluents. *Anal Chem, 38*, 1549-1553.
4. Matthews, D.E.; Bier, D.M. **1983**. Stable isotope methods for nutritional investigation. *Annu Rev Nutr, 3*, 309-339.
5. Matthews, D.E.; Hayes, J.M. **1978**. Isotope-ratio-monitoring gas chromatography-mass spectrometry. *Anal Chem, 50,* 1465-1473.
6. MacCoss, M.J.; Matthews, D.E. **2005**. Quantitative mass spectrometry for proteomics: Teaching a new dog old tricks. *Anal Chem, 77,* 294A-302A.
7. Ong, S.E.; Blagoev, B.; Kratchmarova, I.; Kristensen, D.B.; Steen. H.; Pandey. A.; Mann, M. **2002**. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Mol Cell Proteomics, 1*, 376-386.
8. Oda, Y.; Huang, K.; Cross, F.R.; Cowburn, D.; Chait, B.T. **1999**. Accurate quantitation of protein expression and site-specific phosphorylation. *Proc Natl Acad Sci USA, 96*, 6591-6. The Rockefeller University, 1230 York Avenue, New York, NY 10021 USA.
9. Wu, C.C.; MacCoss, M.J.; Howell, K.E.; Matthews, D.E.; Yates, III, J.R. **2004**. Metabolic labeling of mammalian organisms with stable isotopes for quantitative proteomic analysis. *Anal Chem, 76,* 4951-4959.
10. Krüger, M.; Moser, M.; Ussar, S.; Thievessen, I.; Luber, C.A.; Forner, F.; Schmidt, S.; Zanivan, S.; Fässler, R.; Mann, M. **2008**. SILAC mouse for quantitative proteomics uncovers kindlin-3 as an essential factor for red blood cell function. *Cell, 134*, 353-364.
11. Krijgsveld, J.; Ketting, R.F.; Mahmoudi, T.; Johansen, J.; Artal-Sanz, M.; Verrijzer, C.P.; Plasterk, R.H.; Heck, A.J. **2003**. Metabolic labeling of *C. elegans* and *D. melanogaste*r for quantitative proteomics. *Nat Biotechnol, 21*, 927-31. Epub 2003 Jul 13.

Related Products

Catalog No.	Description
DLM-349	D-Glucose (6,6-D_2, 99%)
DLM-2062	D-Glucose (1,2,3,4,5,6,6-D_7, 98%)
NLM-202	Glycine (^{15}N, 98%)
CLM-468	L-Leucine (1-^{13}C, 99%)
NLM-233	Urea ($^{15}N_2$, 98%+)

"We have been working together with CIL for over 10 years now and are using a great palette of their products with great satisfaction. The high quality and purity of their stable isotope-labeled products combined with their professionalism in handling our requests makes CIL one of the most pleasant companies to work with for a protein analysis facility."

Dr. Marc Moniatte
Head of the EPFL Proteomics Core Facility
Lausanne, Switzerland

Early Stable Isotope Labeling in Proteomics

Timothy D. Veenstra, PhD

- **SILAC**
- **SILAM**
  - MouseExpress® Mouse Feed
  - MouseExpress® Mouse Tissue
- **Peptide Synthesis**
- **Chemical Tagging**
- **Cell Growth Media**
- **Enzymatic Labeling**

Mass Spectrometry Signal Calibration for Protein Quantitation

Michael J. MacCoss, PhD

Early Stable Isotope Labeling in Proteomics

Timothy D. Veenstra, PhD
Laboratory of Proteomics and Analytical Technologies
Frederick National Laboratory for Cancer Research, Frederick, MD 21702-1201 USA

Proteomics, the analysis of the proteins expressed by a cell, tissue or organism under a specific set of conditions, continues to see tremendous growth in sample preparation and instrumental technologies. Proteomic studies are typically designed to analyze thousands of proteins in a single analysis and provide a global, dynamic view of changes in protein expression. While proteomics is formally defined as the complete characterization of the protein complement of a cell, including post-translational modifications, much of the effort has been focused on methods to measure changes in relative protein abundances between distinct cell systems.

While changes in protein expression have typically been studied by separating samples of interest using two-dimensional polyacrylamide gels (2D-PAGE) followed by comparing the intensity of the stained spots between gels, this method has many deficiencies related to reproducibility, proteome coverage and quantitation. Fortunately, there have been several recent developments in the use of stable isotope-labeling strategies that allow the comparison of isotopically distinct proteome samples.

While mass spectrometry has not been historically used for measuring relative protein abundances, stable isotope-labeling methods now make this scenario feasible at both the intact protein and peptide level.[1,2] One of the earliest demonstrations of isotopic-labeling strategies for whole proteomes was studying cadmium (Cd^{2+}) stress response in *Escherichia coli*. *E. coli* was grown in both normal (*i.e.* natural isotopic abundance) and rare isotope (^{13}C, ^{15}N) depleted media.[1] Relative protein abundances were measured by removing equal aliquots of cells from the unstressed (normal medium) and stressed (depleted medium) cultures at different time intervals after Cd^{2+}-addition. The aliquots were combined and the extracted proteins were analyzed using capillary isoelectric focusing coupled on-line with Fourier transform ion cyclotron resonance (FTICR) MS. Cells have also been cultured in ^{15}N-enriched medium and combined with cells cultured in normal medium and differences in peptide abundances measured by proteolytic digesting the intact proteins.[2] In both of these metabolic-labeling methods, isotopically distinct versions of each protein (or peptide) are observed and their relative abundances are quantified by comparing observed peak intensities of each species in the mass spectra, as shown in Figure 1.

While the metabolic-labeling method described above is limited to cells that can be cultured in specifically formulated media, chemical-labeling methods have been developed that are applicable to proteome samples isolated from any conceivable source. One of the earliest developments in the use of stable isotope labeling to quantify changes in the expression of proteins in proteome studies was the isotope-coded affinity tags (ICAT) method.[3] In ICAT labeling, shown in Figure 2, proteins are modified with a reactive group that covalently modifies Cys residues. The ICAT reagent also contains a biotin tag, allowing the modified Cys-containing peptides to be isolated using immobilized avidin. Changes in the relative abundance of peptides from distinct proteome samples is accomplished by the use of isotopically distinct versions of the ICAT reagent – a light isotopic version and a heavy isotopic version in which eight protons in the linker region between the thiol reactive group and the biotin moiety of the ICAT reagent have been substituted with eight deuterons. ICAT labeling results in both stable isotope-labeled Cys-polypeptides, which can aid identification by providing an additional Cys sequence constraint, and provides a significant reduction in complexity of the mixture being analyzed.

To demonstrate the ICAT strategy, a protein extract from cultured mouse B16 melanoma cells was divided into two equal aliquots. One aliquot was derivatized with the light isotopic version of the ICAT-D0 reagent and the other using the ICAT-D8 reagent. The proteomes were pooled, digested with trypsin and labeled Cys-polypeptides isolated. The peptide mixture was analyzed in a single capillary LC/MS experiment. In this analysis, hundreds of pairs of Cys-polypeptides with the expected integral mass difference of 8.0 Da were observed. A few of these peptides are shown in Figure 3. The average ratio of peak areas for the distinct isotopically labeled versions of each peptide was ~ 1.01. Since identical aliquots of the proteome sample were used in this experiment, average ratio of peak areas for the distinct isotopically labeled versions of each peptide was ~ 1.01, consistent with the expected results.

Since these early developments, there have been a number of improvements in isotope-labeling strategies. Metabolic labeling has advanced to the stage at which isotopically labeled mice can be produced through feeding a specialized diet containing stable isotopes of specific essential amino acids. Chemical labeling methods, such as iTRAQ, enable up to eight samples

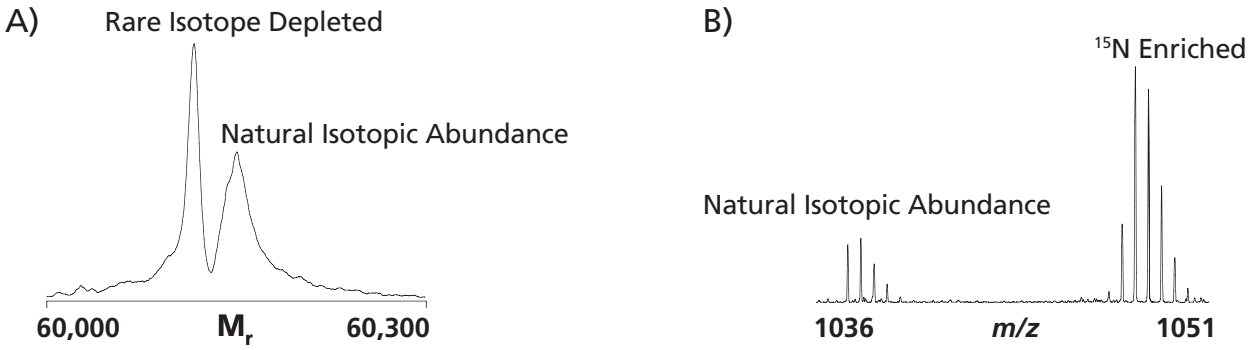


Figure 1. Examples of stable-isotope labeling of an (A) intact protein and (B) peptide observed in the MS analysis of an *E. coli* and *Deinococcus radiodurans* proteome samples, respectively. The two isotopic versions of each were obtained by culturing the cells separately in normal and either isotopically depleted (A) or ^{15}N-enriched (B) media. Combining the two separate cultures provides two isotopic versions for every species present in the samples.

to be compared in a single LC/MS analysis. If methods such as iTRAQ were combined with metabolic labeling, it may be possible to increase this number to 16 or 24 concurrent comparisons.

While stable isotope-labeling methods have been used primarily to measure relative abundance changes of proteins, other strategies have been developed to quantify changes in the phosphorylation state of proteins. The "phosphoprotein isotope-coded affinity tag" (PhIAT) approach differentially labels phosphoseryl (pSer) and phosphothreonyl (pThr) residues with a stable isotopic and biotinylated tag, as shown in Figure 4.[5] This strategy enriches the phosphoprotein pool and enables a quantitative measurement of phosphorylation between the two distinct protein samples by comparison of the extent of isotopic enrichment. After chemically blocking cysteinyl sulfhydryls, phosphoproteins are selectively modified by removing the phosphate group from pSer and pThr residues via hydroxide ion mediated β-elimination. Michael addition to the newly formed α,β-unsaturated residues is performed using 1,2-ethanedithiol (EDT) containing either four alkyl hydrogens (EDT-D) or deuteriums (EDT-D) to achieve stable isotopic labeling. The sulfhydryl groups present of the labeled proteins are biotinylated using iodoacetyl-PEO-biotin to generate PhIAT-labeled proteins. The PhIAT-labeled proteins are digested with trypsin and isolated using immobilized avidin prior to LC/MS analysis. The resultant spectra show two isotopically distinct versions of the same phosphopeptide allowing changes in the peptide's phosphorylation state to be quantified. Successful PhIAT labeling of a control phosphoprotein, as well as proteins from a yeast extract, was demonstrated.

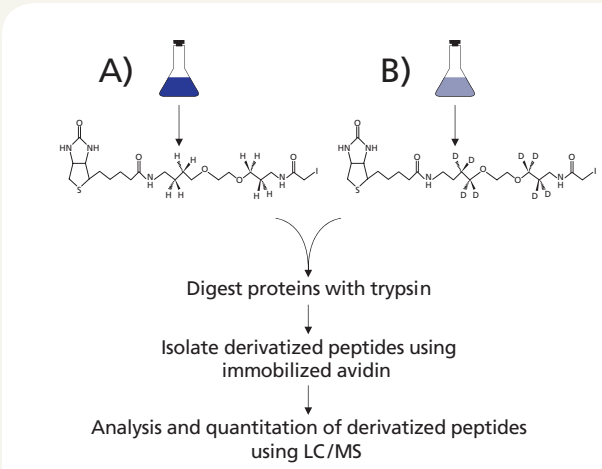


Figure 2. Schematic representation of the isotope-coded affinity tag (ICAT) strategy. Proteins are separately extracted from cells grown under two different conditions (A and B). The proteins for each sample are labeled either with the light (ICAT-D0) or heavy (ICAT-D8) ICAT reagent. After labeling the proteins are pooled and digested with trypsin. The modified peptides are isolated by affinity chromatography and analyzed by capillary LC/MS.

The above presents only a glimpse into the several different types of stable isotope-labeling techniques that are being utilized in proteomics. As this field continues to develop, a variety of stable isotope-labeling methods are being produced. The use of stable isotope-labeling methods to identify and quantify post-translational modifications will become an area of particular importance and growth.

(continued)

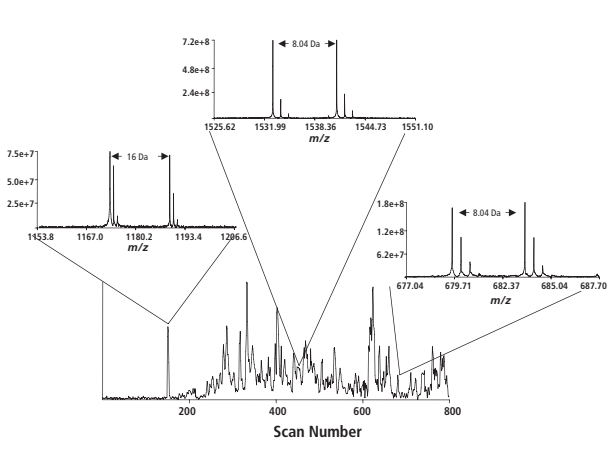


Figure 3. Examples of ICAT-labeled peptides observed in the analysis of mouse B16 melanoma cells. In this analysis, a single proteome sample extracted from the cells was split into two equal aliquots that were then labeled with either ICAT-D0 or ICAT-D8.

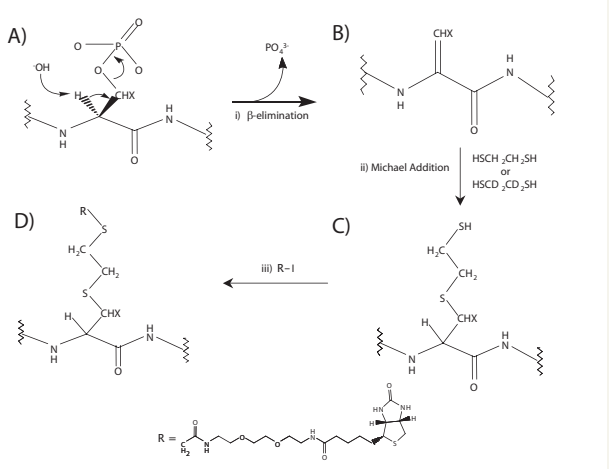


Figure 4. Phosphoprotein isotope-coded affinity tag (PhIAT) labeling method. Proteins containing phosphoseryl (X = H) or phosphothreonyl (X = CH_3) residues are isotopically labeled and biotinylated. After proteolytic digestion, these biotinylated peptides are isolated from non-phosphorylated peptides via avidin affinity chromatography. The ability to quantitate the extent of phosphorylation between two identical peptides extracted from different sources is based on the use of a light ($HSCH_2CH_2SH$, EDT-D_0) and heavy ($HSCD_2CD_2SH$, EDT-D_4) isotopic versions of 1,2-ethanedithiol.

References

1. Pasa-Tolic, L.; Jensen, P.K.; Anderson, G.A.; Lipton, M.S.; Peden, K.K.; Martinovic, S.; Tolic, N.; Bruce, J.E.; Smith, R.D. **1999**. High throughput proteome-wide measurements of protein expression using mass spectrometry. *J Am Chem Soc, 121,* 7949-7950.
2. Oda, Y.; Huang, K.; Cross, F.R.; Crowburn, D.; Chait, B.T. **1999**. Accurate quantitation of protein expression and site-specific phosphorylation. *Proc Natl Acad Sci USA, 96*, 6591-6596.
3. Gygi, S.P.; Rist, B.; Gerber, S.A.; Turecek, F.; Gelb, M.H.; Aebersold, R. **1999**. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nat Biotechnol, 17*, 994-999.
4. Conrads, T.P.; Alving, K.; Veenstra, T.D.; Belov, M.E.; Anderson, G.A.; Anderson, D.J.; Lipton, M.S.; Pasa-Tolic, L.; Udseth, H.R.; Chrisler, W.B.; Thrall, B.D.; Smith, R.D. **2001**. Quantitative analysis of bacterial and mammalian proteomes using a combination of cysteine affinity tags and ^{15}N-metabolic labeling. *Anal Chem*, *73*, 2132–2139.
5. Goshe, M.B.; Conrads, T.P.; Panisko, E.A.; Angell, N.H.; Veenstra, T.D.; Smith, R.D. **2001**. Phosphoprotein isotope-coded affinity tag (PhIAT) approach for isolating and quantitating phosphopeptides in proteome-wide analyses. *Anal Chem, 73*, 2578-2586.

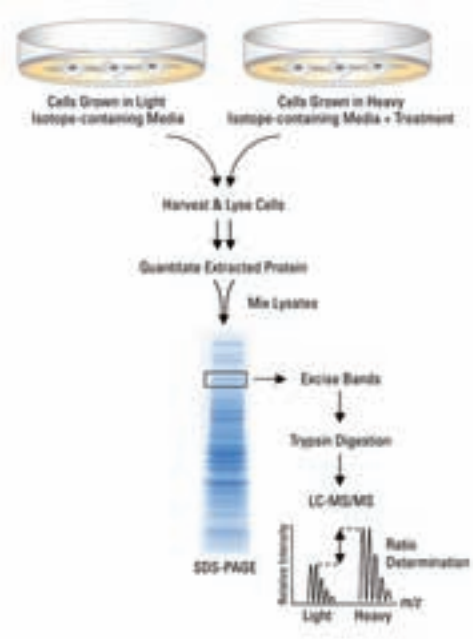
Related Product

Catalog No.	Description
DLM-6785	1,2-Ethanedithiol (1,1,2,2-D_4,98%)

Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC)

Akhilesh Pandey, PhD
Johns Hopkins University, Baltimore, MD 21218 USA

Stable isotope labeling with amino acids in cell culture (SILAC) is a simple and straightforward approach for *in vivo* incorporation of a label into proteins for mass spectrometry (MS)-based quantitative proteomics. SILAC relies on metabolic incorporation of a given "light" (unlabeled) or "heavy" (labeled) form of the amino acid into the proteins. The method relies on the incorporation of amino acids with substituted stable isotopic nuclei (*e.g.* ^{13}C, ^{15}N). Thus, in an experiment, two cell populations are grown in culture media that are identical except that one of them contains a "light" and the other a "heavy" form of a particular amino acid (*e.g.* ^{12}C and ^{13}C labeled L-Lysine, respectively). When the labeled analog of an amino acid is supplied to cells in culture instead of the natural amino acid, it is incorporated into all newly synthesized proteins. After a number of cell divisions, each instance of this particular amino acid will be replaced by its isotope-labeled analog. Since there is hardly any chemical difference between the labeled amino acid and the natural amino acid isotopes, the cells behave exactly like the control cell population grown in the presence of normal amino acid. It is efficient and reproducible as the incorporation of the isotope label is 100%. We anticipate that potential applications of SILAC will lead to its use as a routine technique in all areas of cell biology. *SILAC literature references are on page 23*



Example of SILAC workflow.

SILAC Highlights:

- **Efficient** –100% label incorporation into proteins of cultured cells
- **Reproducible** – eliminates experimental variability caused by differential sample preparation
- **Flexible** – media deficient in both L-Lysine and L-Arginine, allowing for better proteome coverage through dual amino acid isotope labeling
- **Compatible** – label proteins expressed in a wide variety of mammalian cell lines adapted to grow in DMEM or RPMI 1640 medium, including HeLa, 293T, COS7, U2OS, A549, A431, HepG2, NIH 3T3, Jurkat and others
- **99% Enriched High Quality Reagents** – Stable isotope-labeled amino acids with 99% isotopic enrichment and 98%+ chemical purity

SILAC Applications:

- Quantitative analysis of relative changes in protein abundance from different cell treatments
- Quantitative analysis of proteins for which antibodies are unavailable
- Protein expression profiling of normal vs. disease cells
- Identification and quantification of hundreds to thousands of proteins in a single experiment

Refer to www.isotope.com for SILAC protocol or scan the QR code at right.

"Using highly enriched materials decreases the amount of unlabeled analog introduced into the mass spectrometer. As a result, using 99% enriched amino acids will improve the accuracy and useful dynamic range for MS-based quantitative proteomic methods compared to using amino acids with lower enrichments."

Michael Burgess
Biochemist III
Broad Institute, Cambridge, MA

SILAC Kits and Reagents

SILAC Protein Quantitation Kits

Catalog No.	Description
DMEM-LYS-C	SILAC Protein Quantitation Kit DMEM (Dulbecco's Modified Eagle Media) Kit contains: • SILAC DMEM Media, 2 x 500 mL • Dialyzed FBS, 2 x 50 mL • L-Lysine•2HCl ($^{13}C_6$, 99%), 50 mg • L-Lysine•2HCl, 50 mg • L-Arginine•HCl, 2 x 50 mg
RPMI-LYS-C	SILAC Protein Quantitation Kit RPMI 1640 Kit contains: • SILAC RPMI 1640 Media, 2 x 500 mL • Dialyzed FBS, 2 x 50 mL • L-Lysine•2HCl ($^{13}C_6$, 99%), 50 mg • L-Lysine•2HCl, 50 mg • L-Arginine•HCl, 2 x 50 mg
DMEM-500	DMEM Media for SILAC (DMEM minus L-Lysine and L-Arginine)
RPMI-500	RPMI 1640 Media for SILAC (RPMI 1640 minus L-Lysine and L-Arginine)
FBS-50	Dialyzed Fetal Bovine Serum

SILAC Protein Quantitation Kits

Media (DMEM or RPMI 1640), Dialyzed FBS and amino acids are also sold separately.

SILAC Media and Dialyzed FBS are manufactured by Thermo Fisher Scientific, Inc. SILAC Media is provided by Thermo Fisher Scientific under license from the University of Washington and protected by US Patent 6,653,076, for research use only.

Arginine

Catalog No.	Description
CLM-1268	L-Arginine•HCl (1-^{13}C, 99%)
CLM-2070	L-Arginine•HCl (guanido-^{13}C, 99%)
CLM-2051	L-Arginine•HCl (1,2-$^{13}C_2$, 99%)
CLM-2265-H	L-Arginine•HCl ($^{13}C_6$, 99%)
DLM-6038	L-Arginine•HCl (<5% D) (4,4,5,5-D_4, 94%)
DLM-541	L-Arginine•HCl (D_7, 98%)
NLM-1267	L-Arginine•HCl (α-^{15}N, 98%+)
NLM-395	L-Arginine•HCl (guanido-$^{15}N_2$, 98%+)
NLM-396	L-Arginine•HCl ($^{15}N_4$, 98%)
CNLM-7819	L-Arginine•HCl (1-^{13}C, 99%; α-^{15}N, 98%)
CNLM-539-H	L-Arginine•HCl ($^{13}C_6$, 99%; $^{15}N_4$, 99%)
CDLM-3789	L-Arginine•HCl (5-^{13}C, 99%; 4,4,5,5-D_4, 95%)
DNLM-7543	L-Arginine•HCl (D_7, 98%; $^{15}N_4$, 98%)
CDNLM-6801	L-Arginine•HCl ($^{13}C_6$, 97-99%; D_7, 97-99%; $^{15}N_4$, 97-99%)
ULM-8347	L-Arginine•HCl (unlabeled)

Leucine

Catalog No.	Description
CLM-2262-H	L-Leucine ($^{13}C_6$, 99%)
DLM-4212	L-Leucine (isopropyl-D_7, 98%)
CNLM-281-H	L-Leucine ($^{13}C_6$, 99%; ^{15}N, 99%)
CDNLM-4280	L-Leucine ($^{13}C_6$, 95-97%; ^{15}N, 96-99%; 2,3,3-D_3, 97%+)

Lysine

Catalog No.	Description
CLM-653	L-Lysine•2HCl (1-^{13}C, 99%)
CLM-633	L-Lysine•HCl (5-^{13}C, 99%)
CLM-632	L-Lysine•2HCl (6-^{13}C, 99%)
CLM-2247-H	L-Lysine•2HCl ($^{13}C_6$, 99%)
DLM-2640	L-Lysine•2HCl (4,4,5,5-D_4, 96-98%)
DLM-2641	L-Lysine•2HCl (3,3,4,4,5,5,6,6-D_8, 98%)
DLM-570	L-Lysine•2HCl (D_9, 98%)
NLM-143	L-Lysine•2HCl (α-^{15}N, 95-99%)
NLM-631	L-Lysine•2HCl (ε-^{15}N, 98%+)
NLM-1554	L-Lysine•2HCl ($^{15}N_2$, 98%+)
CNLM-7821	L-Lysine•2HCl (1-^{13}C, 99%; α-^{15}N, 98%)
CNLM-3454	L-Lysine•2HCl•H_2O (6-^{13}C, 99%; ε-^{15}N, 98%)
CNLM-291-H	L-Lysine•2HCl ($^{13}C_6$, 99%; $^{15}N_2$, 99%)
DNLM-7545	L-Lysine•2HCl (D_9, 98%; $^{15}N_2$, 98%)
CDNLM-6810	L-Lysine•2HCl ($^{13}C_6$, 97-99%; D_9, 97-99%; $^{15}N_2$, 97-99%)
ULM-8766	L-Lysine•2HCl (unlabeled)

See pages 130-134 for a complete list of amino acids.

NeuCode™ SILAC

Although SILAC has enjoyed great success as a quantitative tool for MS-based proteomics, it does fall short of the higher levels of multiplexing that are capable using well-known isobaric tagging reagents, such as TMT® and iTRAQ. In fact, SILAC is limited in analyzing two, or at best, three samples simultaneously, thereby making SILAC, although information-rich, an inherently low-throughput technique. Fortunately, metabolic labeling in cell culture has recently been profoundly improved with the advent of NeuCode™ SILAC.[1] NeuCode™ SILAC exploits the subtle mass differences in common stable isotopes, *i.e.* mass defect, which ultimately allows for higher levels of multiplexing than conventional SILAC. NeuCode™ SILAC requires specially chosen isotopologues of lysine and other SILAC-type amino acids so that individual peptide signals become revealed under high mass resolving power (>100,000), thereby providing quantitative data within a very small mass space (<40 mDa). Under routine resolution settings, however, the quantitative data is concealed and spectral complexity is markedly reduced compared to traditional SILAC. As new NeuCode™ SILAC amino acid isotopologues become available, the multiplexing capabilities of SILAC will continue to increase.

References

1. Hebert, A.S.; Merrill, A.E.; Bailey, D.J.; Still, A.J.; Westphall, M.S.; Strieter, E.R.; Pagliarini, D.J.; Coon, J.J. **2013**. *Journal Nat Methods*, doi: 10.1038/23nmeth.2378 (epub ahead of print)

SILAC Literature References

Ong, S.E.; Blagoev, B.; Kratchmarova, I.; Kristensen, D.B.; Steen. H.; Pandey. A.; Mann, M. **2002**. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Mol Cell Proteomics, 1*, 376-386.

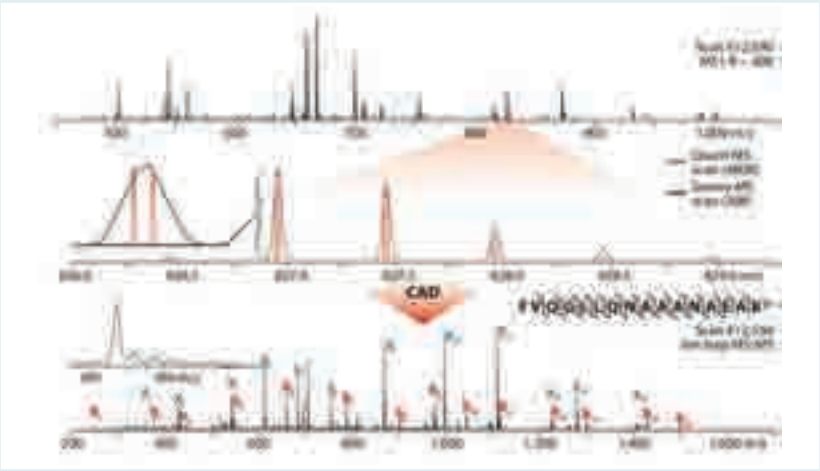
Amanchy, R.; Kalume. D.E.; Pandey, A. **2005**. Stable isotope labeling with amino acids in cell culture (SILAC) for studying dynamics of protein abundance and posttranslational modifications. *Sci STKE, 267,* 1-20.

Blagoev, B.; Ong, S.E.; Kratchmarova. I.; Mann. M. **2004**. Temporal analysis of phosphotyrosine-dependent signaling networks by quantitative proteomics. *Nature Biotechnology, 22*, 1139-1145.

Kratchmarova, I.; Blagoev. B.; Haack-Sorensen. M.; Kassem, M.; Mann, M. **2005**. Mechanism of divergent growth factor effects in mesenchymal stem cell differentiation. *Science, 308*, 1472-1477.

Mann, M. **2006**. Functional and quantitative proteomics using SILAC. *Nat Rev Mol Cell Biol, 7*, 952-958.

Selbach, M.; Mann, M. **2006**. Protein interaction screening by quantitative immunoprecipitation combined with knockdown (QUICK). *Nature Methods, 3*, 981-983.



An MS1 spectrum collected with 30,000 resolving power (top and middle black trace) from an nLC/MS/MS analysis of yeast LysC peptides and a selected precursor having m/z at 827 (middle trace, insert). The insert shows the SILAC pair is concealed at typical resolution. The signal recorded in a subsequent 480,000 resolving power MS1 scan (middle red trace) reveals the quantitative data. MS1 scan of an MS/MS spectrum following CAD and ion trap m/z analysis of the neutron encoded SILAC pair (bottom trace).

Figure provided courtesy of Dr. Joshua J. Coon, Professor of Chemistry and Biomolecular Chemistry, University of Wisconsin-Madison.

"We have enjoyed working together with CIL to develop the NeuCode™ SILAC technology. NeuCode will greatly expand the plexing capacity of SILAC. CIL's technical mastery of how to incorporate isotopes into amino acids has been key to the development of this technology, and we look forward to its continued expansion."

Dr. Joshua J. Coon
Professor of Chemistry and Biomolecular Chemistry
University of Wisconsin-Madison

18-plex SILAC, and there's room to grow. Christopher M. Rose[1]; Alexander S. Hebert[1]; Anna E. Merrill[1]; Derek J. Bailey[1]; Joel C. Bradley[2]; William W. Wood[2]; Marwan Elmasri[2]; Michael S. Westphall[1]; Joshua J. Coon[1].
1. University of Wisconsin, Madison, WI. 2. Cambridge Isotope Laboratories, Inc., Andover, MA.
61ST ASMS Conference, June 9 - 13, 2013, Minneapolis, MN.

Stable Isotope Labeling in Mammals (SILAM)

John R. Yates, III, PhD
Ernest W. Hahn Professor
The Scripps Research Institute, Chemical Physiology & Cell Biology, La Jolla, CA 92037 USA

As Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC) is limited to cell culture, investigations at the tissue, organ or whole animal level require a different methodology. Stable Isotope Labeling in Mammals (SILAM) has been achieved utilizing ^{13}C as well as ^{15}N. Spirulina whole cells (lyophilized powder) (U-^{15}N, 98%+) (NLM-8401) have been used to uniformly label the proteome with ^{15}N. Animal models of human disease provide a powerful system for the study of molecular mechanisms associated with disease. A quantitative proteomic method for the study of *in vivo* biology using ^{15}N Stable Isotope Labeling in Mammals (SILAM) has been created through stable isotope labeling of the rats and mice.[1] Food is prepared using the algae *Arthrospira platensis* (also commonly called spirulina) grown on ^{15}N to incorporate the stable isotope into all proteins. By combining ^{15}N spirulina with a protein-free chow, food is created that provides only ^{15}N-labeled protein with the other nutrients and vitamins required for normal growth.[1-3] This method of stable isotope labeling uses the synthetic machinery of the cell to incorporate ^{15}N into proteins, and as a result it is a comprehensive technique for cell and tissue labeling. Labeled tissues can then be used as an internal standard when mixed with the diseased tissues from an animal model for a disease.

By using "shotgun proteomics" (Figure 1), mixtures of the intact proteins are proteolytically digested and then analyzed by two-dimensional liquid chromatography coupled to a tandem mass spectrometer.[4] A tandem mass spectrometer can rapidly analyze peptides by generating fragmentation patterns for individual peptides in the mixture. Tandem mass spectra collected for peptides are then used as an "address" or "zip code" to identify proteins in sequence databases.[5] Peptides serve as a surrogate for the intact proteins and are used to identify a protein's presence, and through the stable isotope-labeling process, to measure changes in protein expression. By introducing ^{15}N-labeled amino acids into proteins, a "heavier" version of a protein is produced that can be readily differentiated from ^{14}N-labeled proteins (*e.g.* light) by a mass spectrometer. Thus, if a "heavy" normal mouse (control) is compared to a "light" diseased mouse, the differences in protein expression between the two can be determined using shotgun proteomics and mass spectrometry. This process will allow the discovery of pathways or processes that are up- or down-regulated as a function of disease. Other interesting experiments can be performed with SILAM such as "pulse-chase" in rats or mice to measure protein longevity – a parameter that may mark proteins as susceptible to damage and thus likely disease-related proteins.[6]

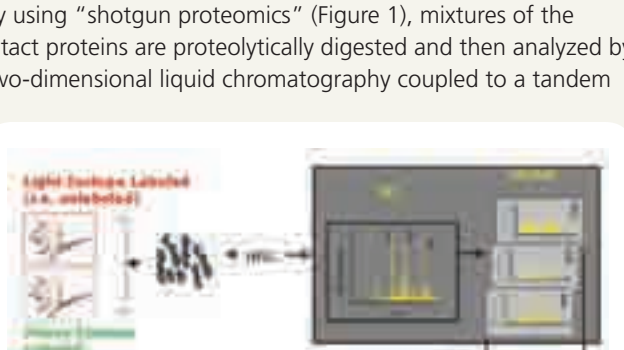
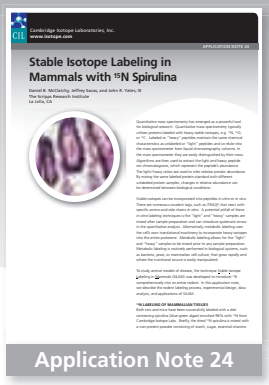


Figure 1. Overview of relative quantitation by stable isotope labeling and mass spectrometry.

References

1. Wu, C.C.; MacCoss, M.J.; Howell, K.E.; Matthews, D.E.; and Yates, J.R., III. **2004**. Metabolic labeling of mammalian organisms with stable isotopes for quantitative proteomic analysis. *Anal Chem, 76,* 4951-4959.
2. McClatchy, D.B.; Dong, M.Q.; Wu, C.C.; Venable, J.D.; Yates, J.R., III. **2007**. ^{15}N metabolic labeling of mammalian tissue with slow protein turnover. *J. Proteome Res, 6*, 2005-2010.
3. McClatchy, D.B.; Liao, L.; Park, S.K.; Venable, J.D.; and Yates, J.R., III. **2007**. Quantification of the synaptosomal proteome of the rat cerebellum during post-natal development. *Genome Res,17,* 1378-1388.
4. Washburn, M.P.; Wolters, D.; Yates, J.R., III. **2001**. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nat. Biotechnol., 19,* 242-247.
5. Sadygov, R.; Cociorva, D.; Yates, J.R., III. **2004**. Large-scale database searching using tandem mass spectra: looking up the answer in the back of the book. *Nature Methods, 1,* 195-202. Res 17, 1378-1388.
6. Savas, J.N.; Toyama, B.H.; Xu, T.; Yates, J.R., III; Hetzer, M.W. **2012**. Extremely long-lived nuclear pore proteins in the rat brain. *Science, 335,* 942.

For a complete listing of MouseExpress® ^{15}N Mouse Feed, please see page 148.



Stable Isotope Labeling in Mammals with ^{15}N Spirulina

Daniel B. McClatchy, Jeffrey Savas, and John R. Yates, III
The Scripps Research Institute, La Jolla, CA 92037 USA

Quantitative mass spectrometry has emerged as a powerful tool for biological research. Quantitative mass spectrometry typically utilizes proteins labeled with heavy stable isotopes, e.g. ^{15}N, ^{18}O, or ^{13}C. Labeled or "heavy" peptides maintain the same chemical characteristics as unlabeled or "light" peptides and co-elute into the mass spectrometer from liquid chromatography columns. In the mass spectrometer they are easily distinguished by their mass. Algorithms are then used to extract the light and heavy peptide ion chromatograms, which represent the peptide's abundance. The light/heavy ratios are used to infer relative protein abundance. By mixing the same labeled protein standard with different unlabeled protein samples, changes in relative abundance can be determined between biological conditions.

Stable isotopes can be incorporated into peptides *in vitro* or *in vivo*. There are numerous covalent tags, such as iTRAQ®, that react with specific amino acid side chains *in vitro*. A potential pitfall of these *in vitro* labeling techniques is the "light" and "heavy" samples are mixed after sample preparation and can introduce systematic errors in the quantitative analysis. Alternatively, metabolic labeling uses the cell's own translational machinery to incorporate heavy isotopes into the entire proteome. Metabolic labeling allows for the "light" and "heavy" samples to be mixed prior to any sample preparation. Metabolic labeling is routinely performed in biological systems, such as bacteria, yeast, or mammalian cell culture, that grow rapidly and where the nutritional source is easily manipulated.

To study animal models of disease, the technique Stable Isotope Labeling in Mammals (SILAM) was developed to introduce ^{15}N comprehensively into an entire rodent. In this application note, we describe the rodent-labeling process, experimental design, data analysis and applications of SILAM.

^{15}N Labeling of Mammalian Tissues

Both rats and mice have been successfully labeled with a diet containing spirulina (blue-green algae) enriched 98% with ^{15}N from Cambridge Isotope Labs. Briefly, the dried ^{15}N spirulina is mixed with a non-protein powder consisting of starch, sugar, essential vitamins and other nutrients. This mixture is kneaded with water to form dough. The dough is then manually shaped into pellets in a similar size to normal rodent diet and dried in a food dehydrator. The only challenge with SILAM is that some tissues are difficult to label (low ^{15}N enrichment <90%) and can result in less efficient and accurate quantitation. Although the nitrogen source is the same for all tissues, the amino acid precursor pools are not. Tissues with slower protein turnover rates, such as brain and muscle, will take longer for the ^{15}N-labeled amino acids to equilibrate with the normal amino acid precursor pool. Initially, experiments were performed using a 1:3 (wt/wt) ratio of ^{15}N Spirulina whole cells (lyophilized powder) (U-^{15}N, 98%+) (NLM-8401) to non-protein powder. In this study, a weaned rat was fed this diet for six weeks. Many tissues were highly enriched (>95%), but tissues with slow protein turnover were not. To obtain high enrichment of these tissues, the diet is fed to a weaned female rat during mating, pregnancy and nursing of its pups. After weaning, the pups are given the ^{15}N diet until postnatal day 45 (p45). At this point, all the tissues have high ^{15}N enrichment. Since mice are more prone to eating their young than rats, generational labeling should be avoided. We have achieved high ^{15}N enrichment in mice by increasing the ^{15}N Spirulina in the diet to a ratio of 1:2, and feeding the mice the ^{15}N diet immediately after weaning for 10 weeks (unpublished data). Although not tested, the 1:2 ratio with the 10-week labeling time should also be sufficient for labeling rats.

For a rat generational labeling experiment, 850 g of ^{15}N Spirulina is required for a typical litter, and for a 10-week mouse labeling experiment, 100 g of ^{15}N Spirulina is required for one mouse.

These ^{15}N labeling protocols result in an average ^{15}N enrichment of at least 95% in all tissues tested. The percent enrichment of the ^{15}N tissues can be predicted from the isotopic distribution of the digested peptides using the algorithm Census.

Experimental Design

When designing SILAM experiments, it is important to remember the ^{15}N tissues are used only as internal standards. To compare two samples, the ^{15}N tissue is mixed with the two samples separately, and then the two $^{14}N/^{15}N$ mixtures are analyzed. Since the ^{15}N tissues are only used as internal standards, this analysis eliminates problems from any potential isotopic effects of the ^{15}N (although we have not observed any adverse biological effects) and corrects for systematic errors that may occur in an experiment. Furthermore, with this experimental design, the ^{15}N internal standard

(continued)

does not need to be identical to the unlabeled samples. For example, we examined differences between the nuclear phosphoproteome of liver and brain tissue using ^{15}N-labeled liver as the internal standard, see Figure 1. In this study, ^{15}N labeled liver homogenate was mixed 1:1 with ^{14}N liver and ^{14}N brain homogenates separately. The nuclei were isolated from the ^{14}N/^{15}N mixtures and then, digested to peptides. The phosphopeptides were enriched using iron metal affinity chromatography (IMAC), and then, the resulting enriched samples were analyzed by Multidimensional Protein Identification Technology (MudPIT). The labeled and unlabeled peptides were identified by SEQUEST, and quantatitive data was calculated by using the Census software.

Data Analysis

The ^{14}N/^{15}N mass spectrometry data can be quantitated using the algorithm Census. An example of the Census output for the phosphorylated peptide, NLAKPGVTSTpSDSEEDDDQEGEK, from the aforementioned liver/brain study is shown in Figure 2. First, the elemental compositions and corresponding isotopic distributions for both the unlabeled and labeled peptides are calculated, and this information is then used to determine the appropriate m/z range for both the ^{14}N and ^{15}N precursor peptides from which to extract the ion chromatograms. Thus, only the identification of the ^{14}N or the ^{15}N peptide is necessary to generate a ^{14}N/^{15}N ratio. Census then calculates the peptide ion intensity ratios for each pair of extracted ^{14}N/^{15}N ion chromatograms by employing a linear least-squares correlation, which is used to calculate the ratio (i.e., slope of the line) and closeness of fit (*i.e.*, correlation coefficient [r]) between the data points of the ^{14}N and ^{15}N ion chromatograms. Census allows users to filter peptide ratio measurements based on the correlation coefficient (values between zero and one). It is recommended accepting only peptide ratios with correlation values greater than 0.5 for accurate quantitation. In addition, Census provides an automated method for detecting and removing statistical outliers using the Grubbs test.

Before comparing the ^{14}N/^{15}N ratios between biological conditions, the data must be normalized. Since the ^{14}N and ^{15}N tissue samples are mixed at a 1:1 ratio, the median of all the ^{14}N/^{15}N peptide ratios within an analysis should be one and if not it is assumed the deviation is due to human error, such as pipetting. Census is able to shift and thus correct the median ^{14}N/^{15}N ratio in order to normalize the data.

It is possible that the difference between a ^{14}N and corresponding ^{15}N peptide is larger than the dynamic range of the mass spectrometer, and this situation is termed a "singleton peptide." As a consequence, a "singleton peptide" is penalized with a low r value and will be filtered out of the dataset. To address this limitation, Census uses a specific linear discriminant analysis to detect "singleton peptides."

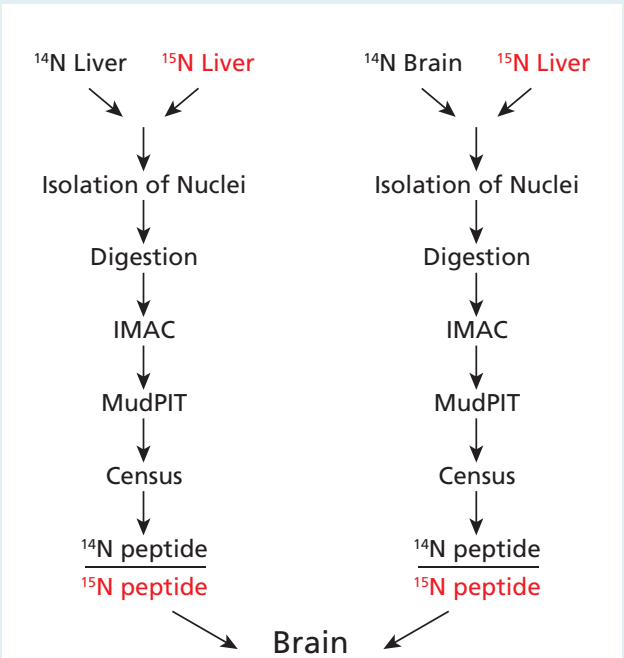


Figure 1. Example of SILAM experiment utilizing ^{15}N liver tissue as an internal standard.

Census is available from the authors for individual use and evaluation through an Institutional Software Transfer Agreement (for details, see http://fields.scripps.edu/census).

SILAM in the Literature

In one report, isolated synapses were quantitated at four developmental time points. For this analysis, the ^{14}N and ^{15}N brain tissue homogenates were mixed prior to the isolation of the synapses. The labeled brain tissue from a p45 rat was employed to quantitate unlabeled brain tissue from rats at p1, p10, p20 and p45, highlighting that accurate and efficient quantitation can be obtained even when the labeled and unlabeled samples are not identical. In this study, the protein expression level of 1138 proteins in four developmental time points were quantitated, and 196 protein alterations were determined to be statistically significant. Over 50% of the developmental changes observed had been previously reported using other protein quantification techniques, and potential novel regulators of neurodevelopment were identified.

In a second report, the phosphoproteome was quantitated from isolated nuclei during brain development. Again, the ^{14}N and ^{15}N tissues were mixed prior to the isolation of the organelle. Although phosphorylation is a reversible and labile modification, a labeled p45 brain successfully quantitated the phosphoproteome at different developmental timepoints. Using IMAC to enrich phosphopeptides from the ^{14}N/^{15}N mixtures, 705 and 1477

phosphopeptides were quantitated from the p1 and p45 brains, respectively. It has been widely observed that different phosphorylation sites on the same protein can be differentially regulated. In this study, differential regulation of phosphorylation sites of methyl-CpG-binding protein 2 (MeCP2) were observed during development. Loss of function mutations in MeCP2 can cause Rett syndrome, a neurodevelopmental disorder, but cellular mechanisms linking the mutations to the phenotype are poorly understood. In a subsequent study, the differential regulation of the phosphorylation sites of MeCP2 identified by SILAM were demonstrated to be crucial for the regulation of transcription by MeCP2.

Related Products

Catalog No.	Description
NLM-8401	Spirulina Whole Cells (lyophilized powder) (U-^{15}N, 98%+)
MLK-LYS-C	Mouse Feed Labeling Kit Kit contains: 1 kg of L-Lysine-$^{13}C_6$ feed and 1 kg of (unlabeled) feed
MF-SPIRULINA-N	MouseExpress® (^{15}N, 98%) Mouse Feed prepared with Spirulina (U-^{15}N, 98%+)
MF-SPIRULINA-N-IR	MouseExpress® (^{15}N, 98%) Mouse Feed, Irradiated prepared with Spirulina (U-^{15}N, 98%+)
MF-SPIRULINA-U	MouseExpress® Unlabeled Mouse Feed prepared with (unlabeled) Spirulina
MF-SPIRULINA-U-IR	MouseExpress® Unlabeled Mouse Feed, Irradiated prepared with (unlabeled) Spirulina
MLK-SPIRULINA-N	MouseExpress® (^{15}N, 98%) Mouse Feed Kit prepared with Spirulina Kit contains: 1 kg Spirulina (^{15}N, 98%) feed and 1 kg Spirulina (unlabeled) feed
MLK-SPIRULINA-N-IR	MouseExpress® (^{15}N, 98%) Mouse Feed Kit, Irradiated prepared with Spirulina Kit contains: 1 kg Spirulina (^{15}N, 98%) feed and 1 kg Spirulina (unlabeled) feed

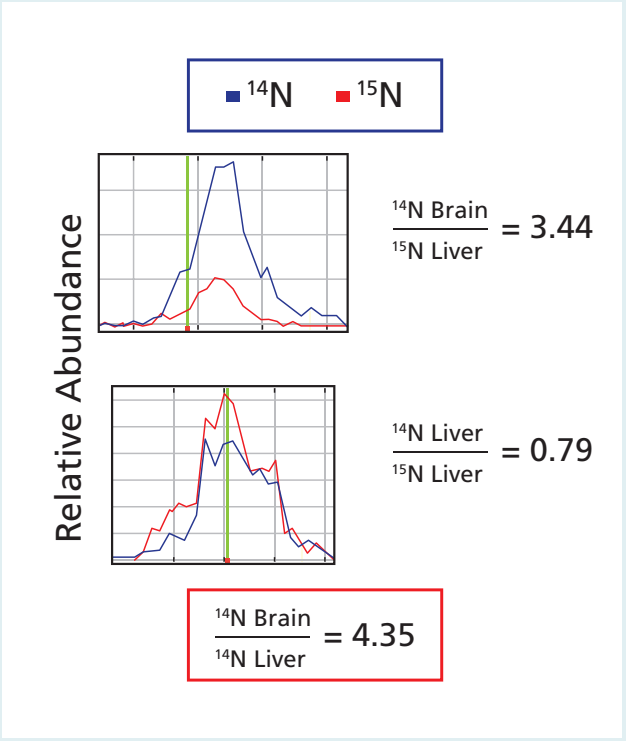


Figure 2. Census output of phosphorylated peptide, NLAKPGVTSTpSDSEEDDDQEGEK.

Please see page 149 for a complete listing of MouseExpress® ^{15}N Mouse Tissue.

References

1. Shadforth, I.P., *et al.* **2005**. i-Tracker: for quantitative proteomics using iTRAQ®. *BMC Genomics, 6*, 145.
2. Wu, C.C., *et al.* **2004**. Metabolic labeling of mammalian organisms with stable isotopes for quantitative proteomic analysis. *Anal Chem, 76*, 4951-9.
3. McClatchy, D.B.; Yates, J.R.I. **2008**. Stable Isotope Labeling of Mammals (SILAM). *CSH Protocols, doi:10.1101/pdb.prot 4940*
4. McNurlan, M.A.; Pain, V.M.; Garlick, P.J. **1980**. Conditions that alter rates of tissue protein synthesis *in vivo*. *Biochem Soc Trans, 8*, 283-5.
5. McClatchy, D.B., *et al.* **2007**. ^{15}N metabolic labeling of mammalian tissue with slow protein turnover. *J Proteome Res, 6*, 2005-10.
6. MacCoss, M.J., *et al.* **2005**. Measurement of the isotope enrichment of stable isotope-labeled proteins using high-resolution mass spectra of peptides. *Anal Chem, 77*, 7646-53.
7. Park, S.K., *et al.* **2008**. A quantitative analysis software tool for mass spectrometry-based proteomics. *Nat. Methods 5, 319-322.*
8. Ficarro, S.B., *et al.* **2002**. Phosphoproteome analysis by mass spectrometry and its application to Saccharomyces cerevisiae. *Nat Biotechnol, 20*, 301-5.
9. Washburn, M.P.; Wolters, D.; Yates, III, J.R. **2001**. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nat Biotechnol, 19*, 242-7.
10. Eng, J.; McCormack, A.L.; Yates, III, J.R. **1994**. An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J Am Soc Mass Spectrom, 5,* 976-989.
11. Ting, L., *et al.* **2009**. Normalization and statistical analysis of quantitative proteomics data generated by metabolic labeling. *Mol Cell Proteomics, 8*, 2227-42.
12. McClatchy, D.B., *et al.* **2007**. Quantification of the synaptosomal proteome of the rat cerebellum during post-natal development. *Genome Res, 17*, 1378-88.
13. Liao, L., *et al.* **2008**. Quantitative analysis of brain nuclear phospho-proteins identifies developmentally regulated phosphorylation events. *J Proteome Res, 7*, 4743-55.
14. Amir, R.E., *et al.* **1999**. Rett syndrome is caused by mutations in X-linked MeCP2, encoding methyl-CpG-binding protein 2. *Nat Genet, 23*, 185-8.
15. Tao, J., *et al.* **2009**. Phosphorylation of MeCP2 at Serine 80 regulates its chromatin association and neurological function. *Proc Natl Acad Sci USA, 106*, 4882-7.

^{15}N Stable Isotope Labeling Data Analysis

Sung Kyu (Robin) Park, CEO Integrated Proteomics Applications, Inc., San Diego, CA 92121 USA
John R. Yates III, PhD The Scripps Research Institute, La Jolla, CA 92037 USA

Census[1] is a freely available quantitative software that can fully analyze ^{15}N-labeled data (http://fields.scripps.edu/).

The Census software provides researchers with unique algorithms such as enrichment ratio calculation, accurate prediction of isotope distribution upon enrichment, sample mixture error correction, outlier filtering and more (Figure 1). SILAM ^{15}N labeling shifts the mass of a peptide based on the number of nitrogen atoms present, which is a function of the amino acid sequence. In order to quantitate peptides, an algorithm needs to be able to calculate the mass shift for every peptide's sequence, which is easy to perform once the sequence is known. Most algorithms do not possess this capability and can only quantitate peptides when there is a set mass shift between peptides. In addition, Census will also calculate the atomic percent enrichment of ^{15}N for every peptide, as this can vary depending on a protein's longevity or turnover rate.

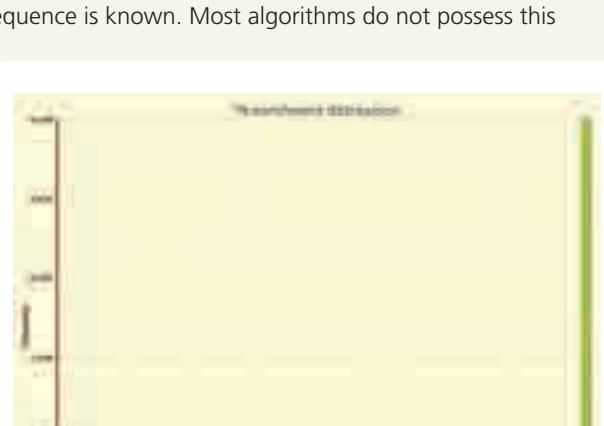


Figure 1. Enrichment calculation.

After protein identification, Census uses the amino acid elemental composition information to calculate corresponding isotopic distributions for both the light and heavy peptides. Ion intensities are extracted from spectral files using a user-defined mass accuracy tolerance to generate chromatograms from the m/z range surrounding both the unlabeled and labeled precursor peptides. Census then calculates peptide ion intensity ratios for each peptide pair using a linear least-squares correlation, which calculates the ratio and closeness of fit between the data points of the unlabeled and labeled ion chromatograms (Figure 2). To determine protein ratios, both mean and weighted means of peptide ratios were calculated upon peptide quality scores.

Integrated Proteomics Pipeline (IP2) is a proteomics data-analysis platform that provides comprehensive proteomics data analysis solutions from protein identification, quantification, modification analysis and multiple experiment analysis (Figure 3). The IP2 software supports the Census software and provides extended data analysis features with graphical tools. IP2 can analyze most quantitative data types, including ^{15}N stable isotope labeling. Users can effortlessly compare multiple samples to find significant proteins through various built-in statistical tools including *t*-test, ANOVA, clustering, post-hoc and more (Figure 4).

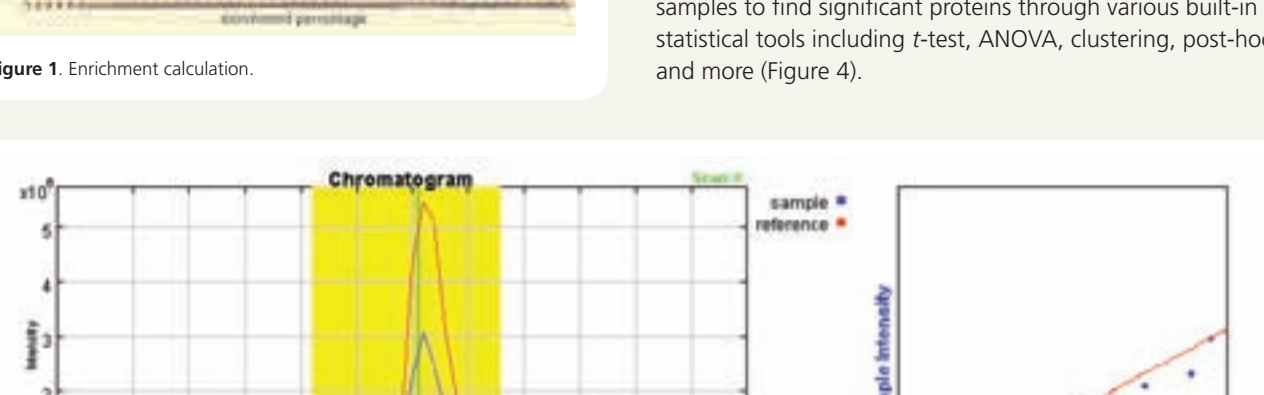


Figure 2. Census ratio calculation.

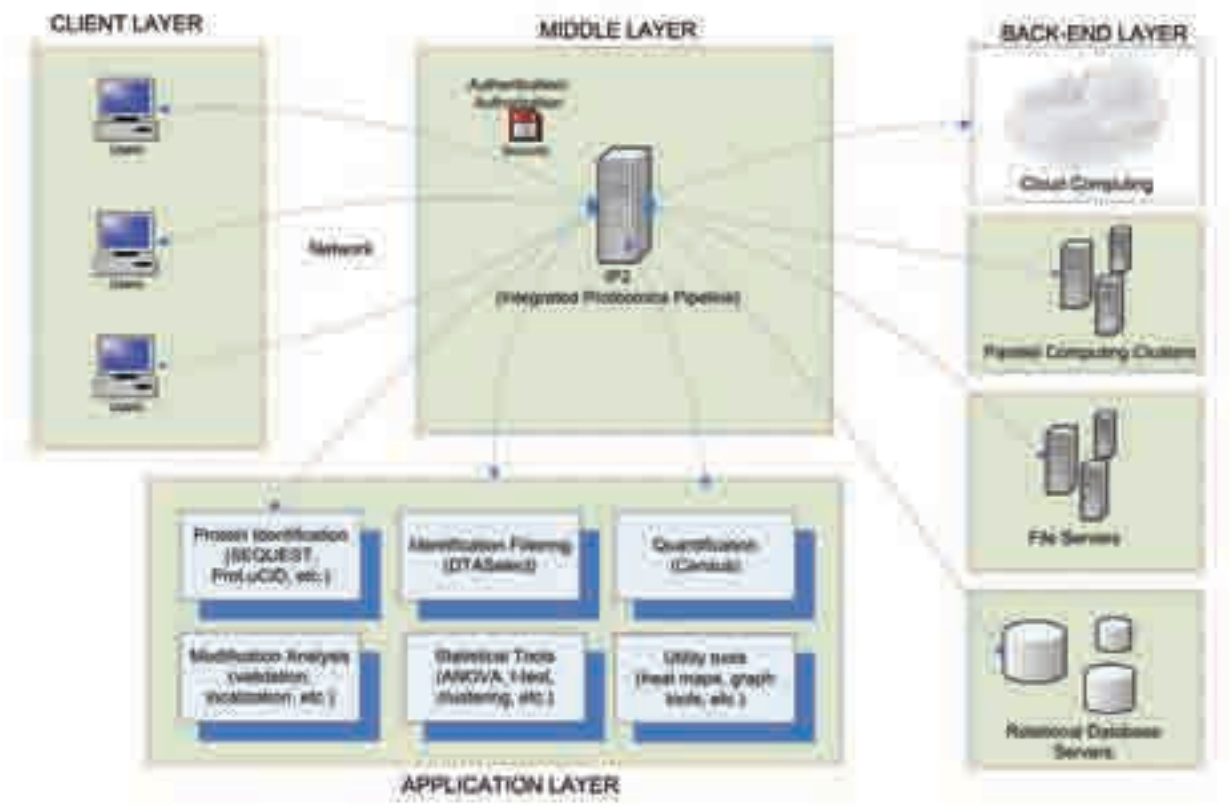


Figure 3. Overview of Integrated Proteomics Pipeline.

Additional information on Integrated Proteomics Pipeline (IP2) can be found at **www.integratedproteomics.com**.

INTEGRATED PROTEOMICS APPLICATIONS, INC.

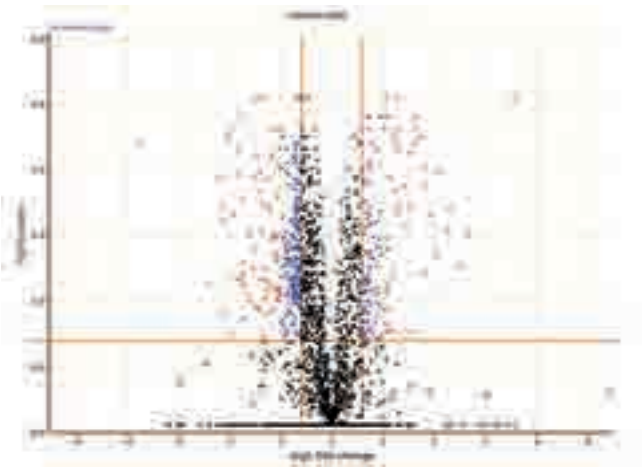


Figure 4. Finding significant proteins through multiple experiment comparison.

References

1. Park, S.K.; Venable, J.D.; Xu, T.; Yates, J.R. III. **2008**. A quantitative analysis software tool for mass spectrometry-based proteomics. *Nat Methods, 5*, 319-22.

Spirulina ^{15}N for SILAM (Stable Isotope Labeling in Mammals)

Spirulina (U-^{15}N, 98%+)

Spirulina (U-^{15}N, 98%+) (NLM-8401), a unique blue-green algae, in combination with a protein/amino acid-free nutrient mix, provides an efficient feed to metabolically label the entire animal proteome with ^{15}N. A ^{15}N rodent diet can be prepared by a custom diet vendor or in your laboratory using ^{15}N Spirulina. Please refer to: McClatchy, D.B. and Yates, J.R., III. **2008**. Stable Isotope Labeling of Mammals (SILAM). Cold Spring Harbor Laboratory. *Protoc, doi:10.*1101/pdb.prot4940

Spirulina cells are grown under carefully controlled conditions in specially designed photobioreactors. Various parameters, such as temperature, pH, O_2 and CO_2 levels are continuously monitored and controlled during the growths. Media and growth conditions have been optimized to give consistently high levels of protein while suppressing algal toxins, such as microcystine.

At harvesting, the Spirulina cells are carefully processed and separated to remove residual media and any other contaminants prior to lyophilization. Each batch of cells is tested by the "Brine Shrimp Test," an industry standard for confirmation of non-toxicity. Our Spirulina cultures are routinely checked by microscopic examination to ensure that the strains are free from other algal contaminants.

CIL can also provide custom prepared diets for your labeling experiments consulting with a custom diet vendor. **Minimum orders may apply.*

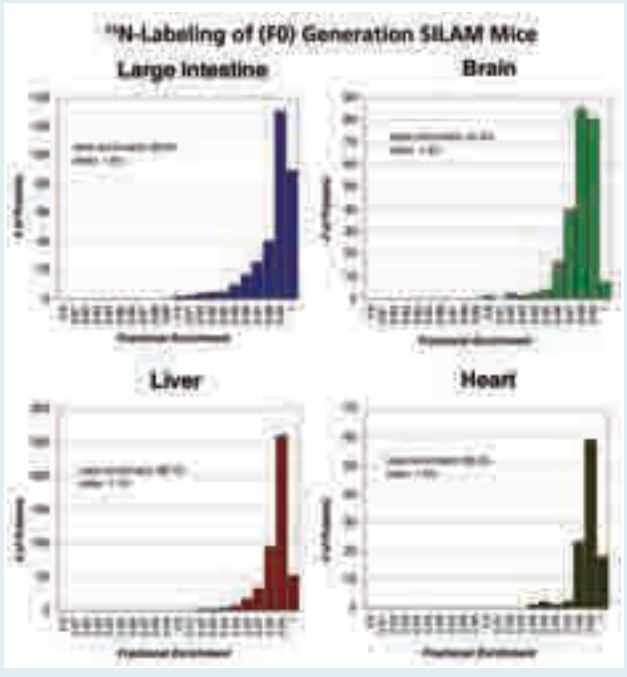
Spirulina

Catalog No.	Description
CLM-8400	Spirulina whole cells (lyophilized powder) (U-^{13}C, 97%+)
NLM-8401	Spirulina whole cells (lyophilized powder) (U-^{15}N, 98%+)
ULM-8453	Spirulina whole cells (lyophilized powder) (unlabeled)

Please see page 148 for a complete listing of MouseExpress® Mouse Feed.

"The use of ^{15}N Spirulina diet for generating SILAM mice was very straightforward and effective. The mice seemed to prefer the Spirulina chow to their normal diet, and behaved and bred normally over multiple generations while consuming this food. Labeling efficiency was very high (~98% enrichment) after only a single generation."

Gabriel M. Simon, PhD
Washington University, Laboratory of Dr. Jeffrey I. Gordon



Graph courtesy of Gabriel M. Simon, PhD

MouseExpress® Mouse Feed

SILAM has also been accomplished using both stable isotope-labeled amino acids as well as ^{15}N Spirulina. CIL is pleased to offer custom feed for the metabolic incorporation of stable isotopes into mice and rats. *Custom formulations are available. Please inquire.*

MouseExpress® (^{15}N, 98%) Mouse Feed

Catalog No.	Description
MF-SPIRULINA-N	MouseExpress® (^{15}N, 98%) Mouse Feed prepared with Spirulina (U-^{15}N, 98%+)
MF-SPIRULINA-N-IR	MouseExpress® (^{15}N, 98%) Mouse Feed, Irradiated prepared with Spirulina (U-^{15}N, 98%+)
MF-SPIRULINA-U	MouseExpress® Unlabeled Mouse Feed prepared with unlabeled Spirulina
MF-SPIRULINA-U-IR	MouseExpress® Unlabeled Mouse Feed, Irradiated prepared with unlabeled Spirulina
MLK-SPIRULINA-N	MouseExpress® (^{15}N, 98%) Mouse Feed Kit prepared with Spirulina Kit contains: 1 kg Spirulina (^{15}N, 98%) feed and 1 kg Spirulina (unlabeled) feed
MLK-SPIRULINA-N-IR	MouseExpress® (^{15}N, 98%) Mouse Feed Kit, Irradiated prepared with Spirulina Kit contains: 1 kg Spirulina (^{15}N, 98%) feed and 1 kg Spirulina (unlabeled) feed

MouseExpress® is a registered trademark of Cambridge Isotope Laboratories, Inc.

Macronutrient information for a representative Spirulina ^{15}N containing diet. Spirulina is sole source of nitrogen.

	% by weight	% kcal from
Protein	18.0	20.0
CHO	53.0	55.0
Fat	10.5	25.0
Kcal/g	**3.8**	

Protein content = N x 6.25, where N = nitrogen content
Macronutrient information provided by Harlan Laboratories, Inc.

MouseExpress® Mouse Feed Labeling Kit

MLK-SPIRULINA-N	MouseExpress® (^{15}N, 98%) Mouse Feed Kit prepared with Spirulina Kit contains: 1 kg Spirulina (^{15}N, 98%) feed and 1 kg Spirulina (unlabeled) feed

Literature References

Butkoa, M.T.; Savas, J.N.; Friedman, B.; Delahunty, C.; Ebner, F.; Yates, J.R. III; Tsien, R.Y. **2013**. *In vivo* quantitative proteomics of somatosensory cortical synapses shows which protein levels are modulated by sensory deprivation. *Proc Natl Acad Sci, USA, 110*, E726-E735.

Savas, J.N.; Toyama, B.H.; Xu, T.; Yates, J.R. III; Hetzer, M.W. **2012**. Extremely Long-Lived Nuclear Pore Proteins in the Rat Brain. *Science, 335*, 942.

Simon, G.M.; Cheng, J.; Gordon, J.I. **2012**. Quantitative assessment of the impact of the gut microbiota on lysine ε-acetylation of host proteins using gnotobiotic mice. *Proc Natl Acad Sci USA, 109,* 11133-11138.

Rauniyar, N.; McClatchy, D.B.; Yates, J.R. III. **2013**. Stable isotope labeling of mammals (SILAM) for *in vivo* quantitative proteomic analysis. *Methods*. [Preprint] Available from http://dx.org/10.1016/j.ymeth.2013.03.00.

MouseExpress® Mouse Feed

MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed

Catalog No.	Description
MF-LYS-C	MouseExpress® L-Lysine ($^{13}C_6$, 98%) Mouse Feed
MF-LYS-C-IR	MouseExpress® L-Lysine ($^{13}C_6$, 98%) Irradiated Mouse Feed
MLK-LYS-C	MouseExpress® L-Lysine ($^{13}C_6$, 98%) Mouse Feed Kit Kit contains: 1 kg of L-Lysine $^{13}C_6$ feed and 1 kg of (unlabeled) feed
MLK-LYS-C-IR	MouseExpress® L-Lysine ($^{13}C_6$, 98%) Mouse Feed Kit, Irradiated Kit contains: 1 kg of L-Lysine $^{13}C_6$ feed and 1 kg of (unlabeled) feed

MouseExpress® Unlabeled Mouse Feed

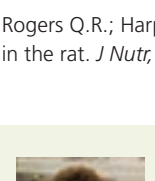
Catalog No.	Description
MF-UNLABELED	MouseExpress® Unlabeled Mouse Feed
MF-UNLABELED-IR	MouseExpress® Unlabeled Irradiated Mouse Feed

Custom-formulated amino acid-defined diets are available in additional labeling patterns and amino acid substitutions. Please inquire.

Diets are designed to meet estimated nutrient requirements for mice and rats.[1] Amino acid pattern adapted from Rogers and Harper.[2]

1 National Research Council. **1995**. *Nutrient Requirements of Laboratory Animals*, Fourth Revised Edition.

2 Rogers Q.R.; Harper, A.E. **1965**. Amino acid diets and maximal growth in the rat. *J Nutr,* 87, 267-273.



"To measure proteome-wide protein turnover in flies and mice we have used a synthetic diet enriched with L-Leucine (5,5,5-D_3) from CIL. The material is extremely cost effective and because of the high frequency of leucine in tryptic peptides, it enables us to use isotopomer distribution analysis to correct for differences in the precursor pool. These are challenging experiments to perform, with lots of details to worry about. Thankfully we have never had to worry about the quality of our stable isotope-labeled material from CIL."

Michael J. MacCoss, PhD
Associate Professor
University of Washington, Dept. of Genome Studies

MouseExpress® L-Leucine (5,5,5-D_3, 98%) Mouse Feed

Catalog No.	Description
MF-LEU-D3	MouseExpress® L-Leucine (5,5,5-D_3, 98%) Mouse Feed
MF-LEU-D3-IR	MouseExpress® L-Leucine (5,5,5-D_3, 98%) Irradiated Mouse Feed
MLK-LEU-D3	MouseExpress® L-Leucine (5,5,5-D_3, 98%) Mouse Feed Kit Kit contains: 1 kg of L-Leucine D_3 feed and 1 kg of (unlabeled) feed
MLK-LEU-D3-IR	MouseExpress® L-Leucine (5,5,5-D_3, 98%) Mouse Feed Kit, Irradiated Kit contains: 1 kg of L-Leucine D_3 feed and 1 kg of (unlabeled) feed

Macronutrient information for an amino acid-defined diet

	% by weight	% kcal from
Protein	14.6	14.9
CHO	65.5	66.8
Fat	8.0	18.4
Kcal/g	**3.9**	

Protein content = N x 6.25, where N = nitrogen content
Macronutrient information provided by Harlan Laboratories, Inc.

Literature References

Dey, A.; Seshasayee, D.; Noubade, R.; French, D.M.; Liu, J.; Chaurushiya, M.S.; Kirkpatrick, D.S.; Pham, V.C.; Lill, J.R.; Bakalarski, C.E.; Wu, J.; Phu, L.; Katavolos, P.; Lafave, L.M.; Abdel-Wahab, O.; Modrusan, Z.; Seshagiri, S.; Dong, K.; Lin, Z.; Balazs, M.; Suriben, R.; Newton, K.; Hymowitz, S.; Garcia-Manero, G.; Martin, F.; Levine, R.L.; Dixit, V.M. **2012**. Loss of the Tumor Suppressor BAP1 Causes Myeloid Transformation. *Science*, *337*, 1541-1546.

Sharma, N.; Medikayala, S.; Defour, A.; Rayavarapu, S.; Brown, K.J.; Hathout, Y.; Jaiswal, J.K. **2012**. Use of quantitative membrane proteomics identifies a novel role of mitochondria in healing injured muscles. *J Biol Chem, 287*, 30455-30467.

Rayavarapu, S.; Coley, W.; Cakir, E.; Jahnke, V.; Takeda, S.; Aoki, Y.; Gordish-Dressman, H.; Jaiswal, J.K.; Hoffman, E.P.; Brown, K.J.; Hathout, Y.; Nagaraju, K. **2013**. Identification of disease specific pathways using *in vivo* SILAC proteomics in dystrophin deficient mdx mouse. *Mol Cell Proteomics.* Jan 7. [Epub ahead of print]

Price, J.C.; Khambatta, C.F.; Li, K.W.; Bruss, M.D.; Shankaran, M.; Dalidd, M.; Floreani, N.A.; Roberts, L.S.; Turner, S.M.; Holmes, W.E.; Hellerstein, M.K. **2012**. The Effect of Long Term Calorie Restriction on *in Vivo* Hepatic Proteostatis: A Novel Combination of Dynamic and Quantitative Proteomics. *Mol Cell Proteomics, 11*, 1801-1814.

MouseExpress® Mouse Tissue

Mass spectrometry methods employing isotope-enriched internal standards have been used quite successfully to quantitatively characterize and compare the proteome from many different cell and tissue types. Quantification of a protein is best achieved by comparing a surrogate peptide with an identical peptide that is labeled with heavy isotopes. A typical quantitative MS-based proteomic workflow will include spiking a known amount of an isotope-enriched peptide to one or more samples to serve as a quantitative internal standard. Unfortunately, in order to simultaneously characterize a large number of proteins within the same sample or across samples, the use of multiple enriched peptides is required which can become prohibitively cumbersome and expensive. To solve this problem, CIL offers MouseExpress® Mouse Tissue, a whole mouse tissue highly enriched with Lysine-$^{13}C_6$ or ^{15}N. The tissue already contains thousands of different heavy-labeled proteins that will yield labeled peptides for use in quantification. MouseExpress® Mouse Tissue allows for an elegant, simultaneous direct comparison of hundreds or even thousands of proteins across samples. MouseExpress® Mouse Tissue may be further processed by the user to enrich certain fractions to simplify the analysis and enhance detectability. MouseExpress® Mouse Tissue may also be used to characterize non-murine, mammalian samples, due to sufficient genetic homology between mice and other mammals.

CIL is pleased to offer intact stable isotope-enriched mouse tissue to assist the MS proteomic community. The use of enriched mouse tissue as an internal standard allows for proteomic investigation at the tissue level[1,2] and will accelerate the quantitative proteome comparison across biological samples.

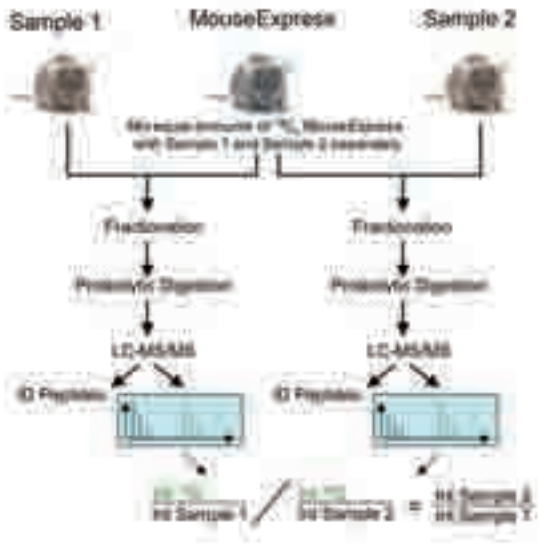
References

1. Boettger, T.; Beetz, N.; Kostin, S.; Schneider, J.; Krüger, M.; Hein, L.; Braun, T. **2009**. Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster. *J Clin Invest, 119*, 2634-2647.
2. McClatchy, D.B.; Dong, M.Q.; Wu, C.C.; Venable, J.D.; Yates, J.R., III. **2007**. ^{15}N metabolic labeling of mammalian tissue with slow protein turnover. *J Proteome Res, 6*, 2005-2010.

"The addition of SILAM standards prepared from CIL's MouseExpress® brain tissue to subcellular fractionation and targeted LC-SRM/MS methodologies represents an essential powerful new tool for the study of neuropsychiatric illness."

Matthew L. MacDonald, PhD
University of Pittsburgh, Translational Neuroscience Program

Proteomic workflow using MouseExpress® L-Lysine ($^{13}C_6$, 97%) mouse tissue



In cooperation with Charles River (GEMS) in Wilmington, MA, Cambridge Isotope Laboratories, Inc., is pleased to offer a SILAM service for the metabolic labeling of mice and rats. Protocols and customized feeds can be created to meet your specific research requirements.

Please inquire for further information or to receive a custom quotation tailored to meet your needs.

MouseExpress® Mouse Tissue

MouseExpress® L-Lysine ($^{13}C_6$, 97%) Mouse Tissue

Product Description

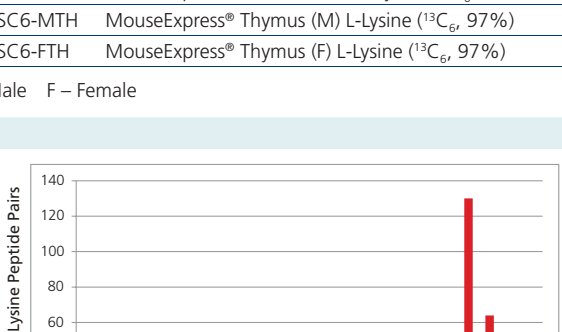
Strain: C57BL6
Form: Intact Tissue
Generation: >F2
Age: Various ages are available; please inquire
Sex: Male or female
Storage: -80° C
Isotope enrichment: $^{13}C_6$ in Lysine >97%

MouseExpress® L-Lysine ($^{13}C_6$, 97%) Mouse Tissue

Catalog No.	Description
MT-LYSC6-MAW	MouseExpress® Abdominal Adipose Tissue (white) (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FAW	MouseExpress® Abdominal Adipose Tissue (white) (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MAB	MouseExpress® Interscapular Adipose Tissue (brown) (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FAB	MouseExpress® Interscapular Adipose Tissue (brown) (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MBL	MouseExpress® Bladder Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FBL	MouseExpress® Bladder Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MBR	MouseExpress® Breast Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FBR	MouseExpress® Breast Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MB	MouseExpress® Brain Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FB	MouseExpress® Brain Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MC	MouseExpress® Cecum Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FC	MouseExpress® Cecum Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MCO	MouseExpress® Colon Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FCO	MouseExpress® Colon Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MD	MouseExpress® Duodenum Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FD	MouseExpress® Duodenum Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MEY	MouseExpress® Eye (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FEY	MouseExpress® Eye (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MFB	MouseExpress® Femur Bone (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FFB	MouseExpress® Femur Bone (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MH	MouseExpress® Heart Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FH	MouseExpress® Heart Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MIL	MouseExpress® Ileum Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FIL	MouseExpress® Ileum Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-ME	MouseExpress® Inner Ear Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FE	MouseExpress® Inner Ear Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MI	MouseExpress® Intestine (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FI	MouseExpress® Intestine (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MJ	MouseExpress® Jejunum Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FJ	MouseExpress® Jejunum Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MK	MouseExpress® Kidney Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FK	MouseExpress® Kidney Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-ML	MouseExpress® Liver Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FL	MouseExpress® Liver Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MLU	MouseExpress® Lung Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FLU	MouseExpress® Lung Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MMAM	MouseExpress® Mammary Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FMAM	MouseExpress® Mammary Tissue (F) L-Lysine ($^{13}C_6$, 97%)

Catalog No.	Description
MT-LYSC6-MM	MouseExpress® Muscle Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FM	MouseExpress® Muscle Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FO	MouseExpress® Ovaries (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MP	MouseExpress® Pancreas Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FP	MouseExpress® Pancreas Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MPL	MouseExpress® Plasma (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FPL	MouseExpress® Plasma (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MSE	MouseExpress® Serum (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FSE	MouseExpress® Serum (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MSK	MouseExpress® Skin Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FSK	MouseExpress® Skin Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MSP	MouseExpress® Spleen Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FSP	MouseExpress® Spleen Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MSC	MouseExpress® Spinal Cord (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FSC	MouseExpress® Spinal Cord (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MST	MouseExpress® Stomach (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FST	MouseExpress® Stomach (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MT	MouseExpress® Testis (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MTB	MouseExpress® Tibia Bone (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FTB	MouseExpress® Tibia Bone (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MTH	MouseExpress® Thymus (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FTH	MouseExpress® Thymus (F) L-Lysine ($^{13}C_6$, 97%)

M – Male F – Female



Incorporation efficiency of Lysine $^{13}C_6$ into peptides extracted from LysC digested mouse blood as determined with a single LC/MS run, by evaluating the ratios between labeled (Lysine $^{13}C_6$) and unlabeled (Lys0) for all detected peptides utilizing MaxQuant.

MouseExpress® Mouse Tissue

MouseExpress® (^{15}N, 94%) Mouse Tissue

Product Description

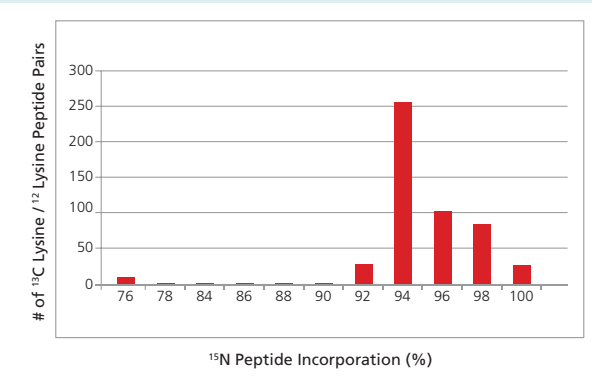
Strain: C57BL6
Form: Intact Tissue
Generation: F0
Age: ~15 weeks
Sex: Male
Storage: -80° C
Isotope enrichment: ^{15}N, 94%

MouseExpress® (^{15}N, 94%) Mouse Tissue

Catalog No.	Description
MT-15N-MB	MouseExpress® Brain Tissue (M) (^{15}N, 94%)
MT-15N-ML	MouseExpress® Liver Tissue (M) (^{15}N, 94%)
MT-15N-MM	MouseExpress® Muscle Tissue (M) (^{15}N, 94%)
MT-15N-MSE	MouseExpress® Serum (M) (^{15}N, 94%)
MT-15N-MSP	MouseExpress® Spleen Tissue (M) (^{15}N, 94%)
MT-15N-MTA	MouseExpress® Tail (M) (^{15}N, 94%)
MT-15N-MT	MouseExpress® Testis Tissue (M) (^{15}N, 94%)

M – Male

Additional tissues available upon request. Please inquire.



MudPIT analysis performed on MouseExpress® ^{15}N brain tissue (F0 generation, 12 weeks of labeling) using an Orbitrap instrument. Both ^{14}N and ^{15}N peptides were searched, and then each ^{14}N corresponding or identified ^{15}N isotopic distribution was compared to theoretical ^{15}N isotopic distributions to calculate ^{15}N peptide enrichment using Census (http://fields.scripps.edu/census) and IP2 (http://www.integratedproteomics.com).

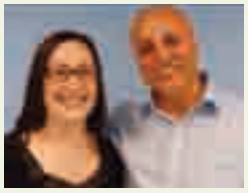
The details of the calculation are in MacCoss, *et. al.*, **2005**. Measurement of the isotopic enrichment of stable isotope-labeled proteins using high-resolution mass spectra of peptides. *Anal Chem, 77*, 7646-53.

"We have worked with CIL for many years and they have been great collaborators. Our interactions with them have been key to helping us develop quantitative proteomic methods, particularly the SILAM technique to study animal models of disease."

John R. Yates, III, PhD
Ernest W. Hahn Professor
The Scripps Research Institute
Chemical Physiology & Cell Biology

Literature Reference

Rauniyar, N.; McClatchy, D.B.; Yates, J.R. III. **2013**. Stable Isotope Labeling of Mammals (SILAM) for *in vivo* quantitative proteomic analysis. *Methods*. pii: S1046-2023(13)00077-7. doi: 10.1016/j.ymeth.2013.03.008. [Epub ahead of print].



We have used the MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed Labeling Kit from Cambridge Isotope Labs (CIL) to label a colony of C57BL6 mice. We achieved full labeling efficiency by F2 generation in the muscle tissue, our tissue of interest, and in all other tissues tested. In addition, CIL's MouseExpress® (^{15}N, 98%) Mouse Feed was used in non-generational labeling of a colony of C57BL6 mice. We achieved full labeling efficiency after 12 weeks of feeding the MouseExpress® (^{15}N, 98%) Mouse Feed. These labeled tissues are fueling a variety of studies for multiple principle investigators at our research institute to study Duchenne muscular dystrophy, myositis, urea cycle disorders and vanishing white matter disease.

Kristy J. Brown, PhD, and Yetrib Hathout, PhD
Children's National Medical Center
Center for Genetic Medicine

Application Note 32

Analysis of Tyrosine Kinase Signaling in Human Cancer by Stable Isotope Labeling with Heavy Amino Acids in Mouse Xenografts Utilizing MouseExpress® Lysine $^{13}C_6$ Mouse Feed

Audrey Sirvent,[1] Serge Urbach,[2] Serge Roche[1]
1. CNRS UMR5237, University of Montpelier 1 and 2, CRBM, 34000 Montpelier, France
2. CNRS UMR5203 INSERM U661, University of Montpelier 1 and 2, IGF, 34000 Montpelier, France

Tyrosine kinases (TK) play important roles in the induction of cell growth, survival and migration. They also have oncogenic activity when deregulated, a role originally described for the constitutively active v-SRC[1] and since then, observed with most TK in human cancer.[2] A large body of evidence indicates that aberrant TK activities contributes to cancer cell growth, survival, angiogenesis and cell dissemination leading to metastasis. This has been illustrated by the capacity of cancer cells transformed by oncogenic TK to induce tumor growth and metastasis formation when injected in nude mice. Since then, they have been considered as attractive therapeutic targets and several inhibitors are currently used in the clinic.[2] However, our knowledge of the TK-dependent oncogenic signaling in human tumors is largely incomplete, mostly because the majority of data has been obtained in two-dimensional cell culture models. Moreover, the standard culture conditions of transformed cells do not allow recapitulating all the kinase-dependent signaling cascades that are activated during tumorigenesis to promote tumor growth, angiogenesis and interactions with the microenvironment.

MS-based quantitative phosphoproteomic technology has been a valuable tool to decipher signaling pathways initiated by a given TK.[3] Particularly, the Stable Isotope Labeling with Amino acids in Cell culture (SILAC) method has been employed for the characterization of oncogenic TK signaling pathways in cell culture.[4, 5] We recently used this powerful approach to investigate oncogenic signaling dependent upon the activity of the TK SRC in colon cancer cells[6] and identified the first SRC-dependent tyrosine "phosphoproteome" in these cancer cells. Oncogenic signaling induced by TK could be investigated *in vivo* using similar MS-based quantitative phosphoproteomic approaches in mouse models or tumor biopsies. However, the application of the SILAC method *in vivo* has been challenging until recently because it requires efficient protein labeling in different tissues, which is conditioned by the rate of *de novo* protein synthesis. Recently, Mann *et al.* described the successful development of a SILAC approach for labeling mice that is based on the addition of L-Lysine•2HCl ($^{13}C_6$, 99%) (CLM-2247-H) into their food.[7] They reported complete labeling from the F2 generation.

In this note, we describe a novel proteomic approach to label tumors in *nude* mice xenografted with human cancer cells using MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed (MF-LYS-C).[8] We reasoned that the high rate of *de novo* protein synthesis occurring in tumors may induce an efficient labeling of xenografted tumors within a short period of time. We observed a consistent >88% labeling of the tumor proteome by feeding engrafted mice with the SILAC mouse diet for only 30 days. We then used this approach to compare the tyrosine phosphoproteome of SRC positive tumors (labeled with heavy amino acids) and of control tumors (labeled with light amino acids).

Experimental Design

Mouse xenografts, [$^{13}C_6$]-Lysine tumor labeling and protein extraction. Swiss *nu/nu (nude)* mice (Charles River, L'Arbresle, France) were injected s.c. with 2x10^6 cells (SRC-SW620 or control SW620 cells) in the flank and fed respectively with L-Lysine $^{13}C_6$ feed or unlabeled feed using MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed Labeling Kit (MLK-LYS-C). After 30 days, animals

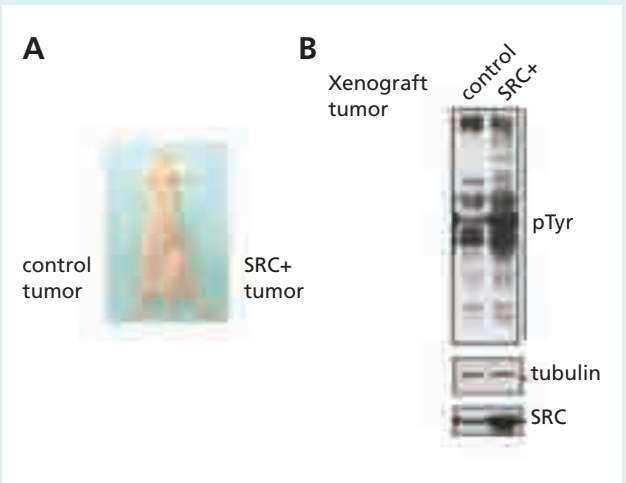


Figure 1. SRC increases tumor growth and pTyr content in CRC xenograft models. A) A representative example of xenograft tumors obtained by subcutaneous injection of controls SW620 CRC cells (left) and SRC-overexpressing SW620 CRC cells (right) in nude mice. B) A representative example of pTyr-level obtained from control and SRC-overexpressing tumor-lysates.

(continued)

were then sacrificed, tumors dissected and protein extracted from frozen tumors using lysis buffer (20 mM Hepes, 150 mM NaCl, 0.5% Triton, 6 mM β-octylglucoside, 100 µM orthovanadate, 100 µM aprotinin, 100 mM DTT, 100 mM NAF) and a Duall® Glass Tissue Grinder size 21.

Mass spectrometry analysis. Phosphotyrosine immunoaffinity purification (using a mixture of 4G10 and pY100 antibodies), and tryptic digestion were essentially performed as described in ref. 9. Purified proteins were separated on 9% SDS-PAGE gels, digested with Lysine C endoproteinase (Thermo Scientific) and analyzed on-line using nanoflow HPLC-nano-electrospray ionization on a LTQ-Orbitrap XL mass spectrometer (ThermoScientific, Waltham, MA USA) coupled with an Ultimate 3000 HPLC apparatus (Dionex, Amsterdam, Netherland). Spectra were acquired with the instrument operating in the information-dependent acquisition mode throughout the HPLC gradient. Survey scans were acquired in the Orbitrap system with resolution set at a value of 60,000. Up to five of the most intense ions per cycle were fragmented and analyzed in the linear trap. Peptide fragmentation was performed using nitrogen gas on the most abundant and at least doubly charged ions detected in the initial MS scan and an active exclusion time of 1 min. Ion selection was set at 5.000 counts.

Data Analysis

Analysis was performed using the MaxQuant software (version 1.1.1.36). All MS/MS spectra were searched using Andromeda against a decoy database consisting of a combination of *Homo sapiens* and *Mus musculus* CPS databases (97,681 entries, release Jun 2011 http://www.expasy.ch) and 250 classical contaminants, containing forward and reverse entities. The statistical validity of the results and the determination of over-represented proteins were assessed using significance B, as defined using Perseus (version 1.1.1.36, standard parameters) on the logarithmized normalized ratio (base 2).

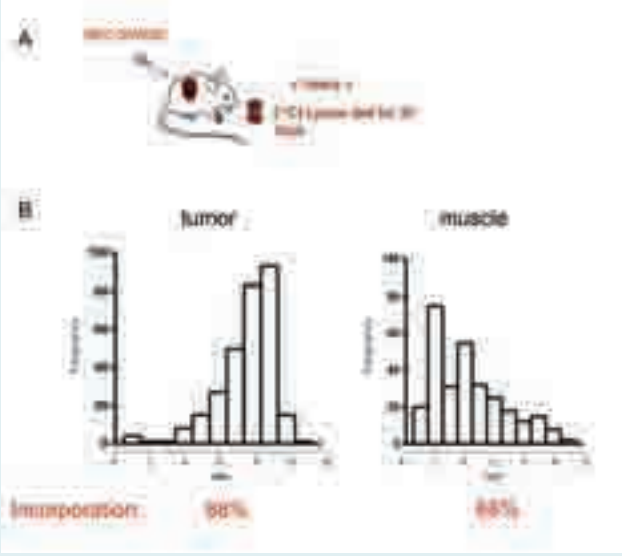


Figure 2. Time course of [$^{13}C_6$]-Lysine incorporation in xenograft tumors. A. Schematic of the analysis of heavy [$^{13}C_6$]-Lysine incorporation in mouse xenograft tumors. After subcutaneous injection of SRC-SW620 cells into the flank of nude mice, animals were subjected to heavy SILAC diet containing [$^{13}C_6$]-Lysine for 30 days. B. Histogram showing the distribution of the incorporation ratios in tumor proteins and in proteins of the muscle tissue surrounding the tumor. The mean ratio incorporation (%) is indicated.

Results

Stable isotope labeling with amino acids in mouse xenografts. Expression of SRC in SW620 cells, a human metastatic colorectal cell line that exhibits a low level of endogenous SRC, increased cell transforming properties as it significantly promoted tumor growth when subcutaneous injected in *nude* mice (Figure 1A). These SRC oncogenic effects were associated with a strong increase of the pTyr content in xenograft tumors in which SRC was overexpressed (Figure 1B). We then applied a MS-based quantitative phosphoproteomic method based on stable isotope labeling with amino acids in mouse xenografts, to thoroughly characterize the SRC-dependent oncogenic signaling pathway in xenograft tumors. Mice were subcutaneously injected with $2x10^6$ SRC-SW620 cells and then fed with MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed, as done to obtain the SILAC mouse, but only during the time required for tumors to reach a volume of about 900 mm^3 (30 days). Tumor proteins were then solubilized from isolated tumors and separated on 1D SDS-PAGE gels, then in-gel digested with the endoproteinase Lys-C and analyzed by liquid chromatography-tandem MS. Digested peptides were then quantified based on the relative Lys intensities. We observed a median SILAC ratio of 1:7.4 at day 30, which corresponded to >88% of tumor protein labeling (Figure 2). These ratios were very consistent over time and in tumors from different animals, further validating our *in vivo* SILAC approach. In contrast, the median SILAC ratio of non-transformed surrounding tissue (*i.e.* muscle) reached 1.97, which corresponded to 66% of protein labeling (Figure 2B). Altogether these results indicate that, while insufficient for labeling non-transformed tissues of the host mice, a 30-day SILAC mouse diet is sufficient to label xenograft tumors to a level that is adequate for quantitative proteomic analysis.

Quantitative phosphoproteomics in xenograft tumors. We next applied this mouse SILAC approach to investigate the SRC-dependent oncogenic signaling pathway in xenografted tumors. SRC-SW620 cells were injected in animals that were fed a diet of MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed. As a control, parental SW620 cells were injected in mice that were fed with a "light" diet of MouseExpress® Unlabeled Mouse Feed (MF-UNLABELED). After 30 days of this regimen, xenograft tumors were isolated and lysed, and three pairs of lysates were prepared by mixing (1:1) one SRC-SW620 xenograft tumor lysate with one control tumor lysate. pTyr proteins were then purified using anti-pTyr antibodies and analyzed by MS.[9] A scheme of the procedure is illustrated in Figure 3A. Quantitative phosphoproteomic analysis led to the identification of 61 SRC targets *in vivo* that were obtained with a ratio significantly >1 in two out of three separate experiments (tyrosine phosphorylated proteins or proteins whose

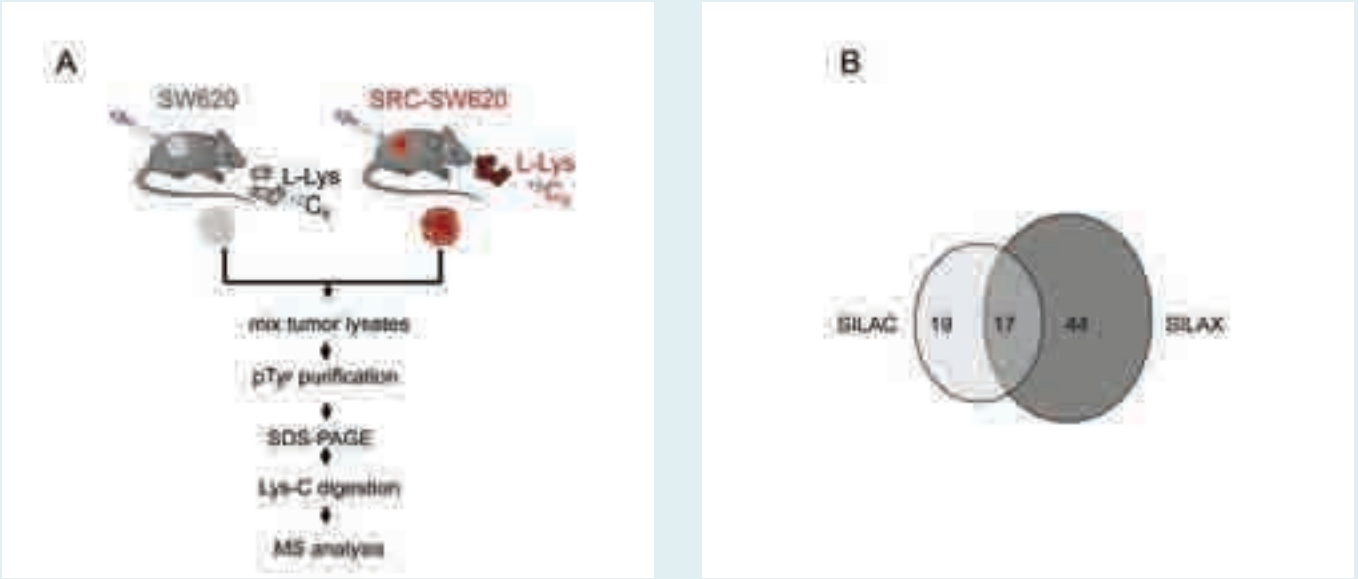


Figure 3. Analysis of SRC oncogenic signaling in xenograft tumors by SILAC mouse. A. Schematic overview of the SILAC experimental procedure applied to mouse xenografts. B. Comparison of *in vivo* and *in vitro* SRC signaling by MS analysis. Venn diagram showing the number of common and specific SRC targets identified by mouse SILAC *in vivo* and SILAC in cell culture.

association with pTyr proteins is increased). A comparison of SRC targets obtained by our *in vivo* analysis with the one obtained by SILAC analysis of the same cancer cells in culture indicates that only 17/61 were also targets of SRC *in vitro* (Figure 3B). This data indicates that oncogenic signaling induced by SRC in tumors significantly differs from the one induced by SRC in cell culture.

Discussion

Here we describe a novel SILAC approach to investigate oncogenic TK signaling *in vivo* in mouse xenografts. This method is based on the efficient labeling of tumor proteins by feeding xenografted mice with the mouse SILAC diet for a limited period of time (30 days) thanks to the high rate of *de novo* protein synthesis in tumors. Indeed, we could successfully label xenograft tumors derived from human colon cells that are characterized by a much slower *in vitro* growth rate than human leukemic cells. Therefore, we think that this approach may be suitable for most human cancer cells that induce significant tumor growth in nude mice. We also predict that our mouse SILAC approach will have a large number of applications, including for the analysis of the dynamic signaling of oncogenic TK during tumor progression from early tumorigenesis to metastasis formation, and also for evaluating the activity of TK inhibitors on the tumor phosphoproteome over time. In this case, this methodology could be particularly useful for determining the molecular cause(s) of innate or acquired resistance to such inhibitors.

Related Products

Catalog No.	Description
MLK-LYS-C	MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed Labeling Kit
MF-LYS-C	MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed
CLM-2247-H	L-Lysine•2HCl ($^{13}C_6$, 99%)

References

1. Yeatman, T.J. **2004**. A renaissance for SRC. *Nat Rev Cancer, 4,* 470-480.
2. Krause, D.S., Van Etten, R.A. **2005**. Tyrosine kinases as targets for cancer therapy. *N Engl J Med, 353*, 172-187.
3. Schmelzle, K., White, F.M. **2006**. Phosphoproteomic approaches to elucidate cellular signaling networks. *Curr Opin Biotechnol, 17,* 406-414.
4. Bose, R.; Molina, H.; Patterson, A.S.; Bitok, J.K.; Periaswamy, B.; Bader, J.S.; Pandey, A.; Cole, P.A. **2006**. Phosphoproteomic analysis of Her2/neu signaling and inhibition. *Proc Natl Acad Sci USA, 103,* 9773-9778.
5. Liang, X.; Hajivandi, M.; Veach, D.; Wisniewski, D.; Clarkson, B.; Resh, M.D.; Pope, R.M. **2006**. Quantification of change in phosphorylation of BCR-ABL kinase and its substrates in response to Imatinib treatment in humanchronic myelogenous leukemia cells. *Proteomics, 6,* 4554-4564.
6. Leroy, C.; Fialin, C.; Sirvent, A.; Simon, V.; Urbach, S.; Poncet, J.; Robert, B.; Jouin, P.; Roche, S. **2009**. Quantitative phosphoproteomics reveals a cluster of tyrosine kinases that mediates SRC invasive activity in advanced colon carcinoma cells. *Cancer Res, 69,* 2279-2286.
7. Kruger, M.; Moser, M.; Ussar, S.; Thievessen, I.; Luber, C.A.; Forner, F.; Schmidt, S.; Zanivan, S.; Fassler, R.; Mann, M. **2008**. SILAC mouse for quantitative proteomics uncovers kindlin-3 as an essential factor for red blood cell function. *Cell, 134,* 353-364.
8. Sirvent, A.; Vigy, O.; Orsetti, B.; Urbach, S.; Roche, S. **2012**. Analysis of SRC oncogenic signaling in colorectal cancer by stable isotope labeling with heavy amino acids in mouse Xenografts. *Mol Cell Proteomics*. 2012 Sep 29. [Epub ahead of print]
9. Amanchy, R.; Kalume, D.E.; Iwahori, A.; Zhong, J.; Pandey, A. **2005**. Phosphoproteome analysis of HeLa cells using stable isotope labeling with amino acids in cell culture (SILAC). *J Proteome Res, 4,* 1661-1671.

Targeted LC-SRM/MS Quantification of Mammalian Synaptic Proteins with MouseExpress® Brain Tissue, a New Isotopically Labeled Proteome Standard

Matthew L. MacDonald,[1*] Eugene Ciccimaro,[2*] Ian Blair,[1] Chang-Gyu Hahn[3]
1. Department of Pharmacology, University of Pennsylvania, Philadelphia, PA
2. ThermoFisher Scientific, Somerset, NJ
3. Department of Psychiatry, University of Pennsylvania, Philadelphia, PA
*Primary Authors

Maintenance and reshaping of synaptic connections in the brain underlies the ability to learn and remember. Complex protein machinery at the synapse is constantly seeking out, strengthening and pruning neuronal connections, literally rewiring in response to experience. The process by which neurons accomplish this feat has been termed "neuroplasticity." Elucidating the molecular mechanics of neuroplasticity is critical to understanding how the human brain interacts with the world around it, as well as how neuropsychiatric diseases assault our memories, thoughts and motor function.

Trafficking of synaptic proteins is critical to neuroplasticity, and many of these trafficked proteins have been implicated in a broad spectrum of neuropsychiatric disorders.[1,2] An in-depth understanding of how these trafficking events function could hold the key to developing new therapeutics. It has become clear that targeted multiplexed quantification of proteins across neuronal subcellular domains is essential to probe these normal and aberrant trafficking events. Mass spectrometry (MS)-based proteomics has emerged as a valuable tool for studying these phenomena. Stable isotope labeling with amino acids in cell culture (SILAC) based methodologies have been successfully applied to cellular models of many diseases.[3] However, this methodology has found limited utility in neuroscience research as complex psychiatric diseases are difficult to model in animals and are impossible to model in culture. Isobaric-labeling methodologies, such as iTRAQ, have proven more useful in assaying trafficking in humans and animal models,[4,5] but peptide chemical tagging methodologies suffer from compression of the quantitative signal and have difficulty repetitively quantifying a targeted subset of peptides. The availability of protein standards, from Cambridge Isotope Labs, generated from Stable Isotope Labeling in Mammals (SILAM) with L-Lysine•2HCl ($^{13}C_6$, 99%) (CLM-2247-H) allows multiplexed targeted quantitative analysisof protein trafficking in brain tissue without the costly and untimely synthesis of individual peptide or protein standards.

In this note, we describe a liquid chromatography-selected reaction monitoring (LC-SRM)/MS approach for the targeted quantification of synaptic peptides in subcellular fractions of mammalian brain tissue utilizing Cambridge Isotope Lab's MouseExpress® Brain Tissue L-Lysine ($^{13}C_6$, 97%) (MT-LYS6-MB). This method utilizes membrane preparations from SILAM brain homogenate as an internal standard facilitating the quantification of over 100 proteins across three neuronal subfractions: vesicular, presynaptic and postsynaptic density, isolated from mouse brain tissue.

Experimental Design

SRM design: Discovery data from 2D LC-MS/MS analyses of synaptic fractions from mouse and human tissue were mined for tryptic peptides from 200 synaptic proteins of interest.[4,6] Pinpoint™ (ThermoFisher Scientific) was utilized to select peptides containing at least one lysine with homology between mouse and human sequences. Peptide homology allows this method to be utilized in clinical subjects as well as additional animal models.

Fractionation and sample preparation: "Light" (unlabeled) vesicular, presynaptic and postsynaptic density fractions were prepared from the cortex of two mice by sucrose density gradient centrifugation and pH-specific Triton X-100 precipitation (Figure 1). "Heavy" (labeled) membrane fractions were prepared from 40 mg MouseExpress® Brain Tissue (male) L-Lysine ($^{13}C_6$, 97%) (MT-LYSC6) using an abbreviated version of the fractionation method (Figure 1). "Light" fractions were mixed with MouseExpress® Brain Tissue preparations at a ratio of 2:1 (Figure 1). The mixed proteomes were separated on a 4-12% bis-Tris gel, divided into two fractions: (*i.e.* <64 kDa and between 64 – 250 kDa, reduced, alkylated and digested with trypsin (Figure 1)).

LC-SMR/MS: The resulting peptides were desalted offline and analyzed on a TSQ Vantage QqQ (ThermoFisher Scientific) interfaced to a NanoLC-Ultra 2D (Eksigent) and CaptiveSpray source (Michrom). A 5 µl peptide sample was loaded onto a 150 mm X 100 µm Magic C18 column (Michrom) at 1 µl/min 97% mobile phase A (H_2O with 0.1% (v/v) formic acid) 3% mobile phase B (acetonitrile with 0.1% (v/v) formic acid) for 12 min, and eluted at 750 nl/min over a 25 min gradient from 3-35% mobile phase B. Retention windows were 1 – 1.5 min, depending on the number of SRMs to be analyzed. In this manner, SRM Dwell times are dynamic, based on the number of analytes eluting at a given time, and range from 5 to 60 msec per transition monitored in these experiments.

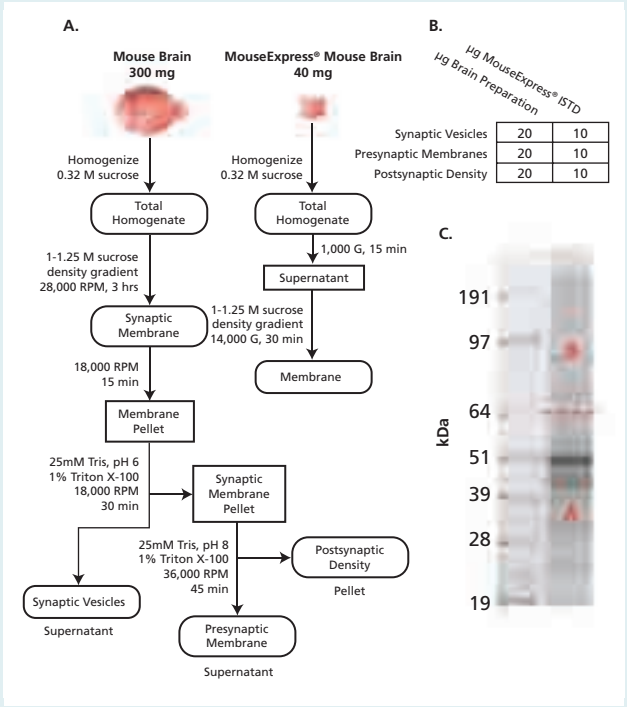


Figure 1. Brain Tissue Fractionation and Sample Preparation:
A) Synaptic vesicle, presynaptic membrane and postsynaptic density enrichments were prepared from 300 mg mouse brain tissue using a variation of the method described in Hahn *et al.* 2009. A membrane fraction was prepared from 40 mg of MouseExpress® Brain Tissue for use as an internal standard (ISTD). **B)** The "light" fractions were mixed 2:1 with the "heavy" MouseExpress® membrane internal standard (ISTD). **C)** The mixed proteomes were separated in the first dimension by gel electrophoresis and divided into two fractions: 19-64 kDa and 64-250 kDa for on-gel trypsin digestion.

Data Analysis

First, "light"/"heavy" ratios were calculated for each peptide in Pinpoint™ using the integrated area under the curve (Figure 2). Where multiple peptides from a single protein were monitored, Pinpoint™ averaged the ratio values. To gain a global view of the data, the proteins were grouped into functional categories by gene ontology (GO) terms within ProteinCenter™ (ThermoFisher Scientific). Relative amounts of representative proteins from four of these categories are reported in Figure 3.

Results

We observed a significant interaction between microdomain partitioning and functions performed in those domains. A subset of the data for four families is reported in Figure 3. The postsynaptic density (PSD) fraction is highly enriched in kinases and scaffolding proteins, such as PSD-95. Scaffolding proteins link glutamate receptors to each other and kinases in PSD facilitating amplification and propagation of neurotransmitter signals. The synaptic vesicles, on the other hand, are highly enriched for soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) complex proteins, responsible for mediating vesicular fusion, and display a moderate enrichment of G-Protein subunits. The SNARE complex is responsible for fusing vesicles to the presynaptic membrane for neurotransmitter release.

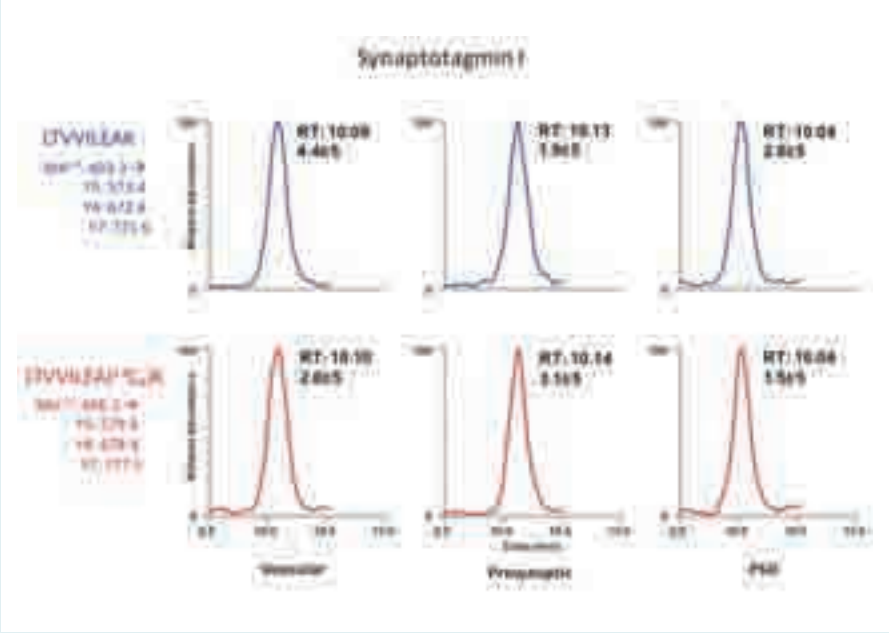
Discussion

Quantitative analyses of subcellular proteomes has become increasingly important in neuroscience. Intracellular signaling pathways in the brain are no longer seen as a linear process from a molecule to the next, but are thought to occur in the context of numerous other protein interactions. This shift in paradigm has begun to impact the study of neuropsychiatric illnesses and thus, it is critically important to have the ability to quantify many proteins simultaneously with microdomain specificity.

The neuronal protein standards prepared from SILAM tissue facilitated the development of a multiplexed assay for the targeted quantification of synaptic proteins. This method represents a significant advance over existing methodologies. Stable isotope-labeled peptide standards may allow for absolute quantification of target proteins and PTMs, but assaying large numbers of proteins can be prohibitively expensive. Furthermore, digestion efficiency and peptide recovery can be difficult to control for using peptide standards. We have found the reproducibility of the LC-SRM/MS with the MouseExpress® Brain Tissue SILAM standard to be superior to quantification with AQUA peptides when samples incur lengthy predigestion sample preparation. Isobaric-labeling methods allow for the quantification of large numbers of proteins, but performing targeted analysis can be difficult and tissue requirements can be high, which is problematic when assaying proteins in discreet rodent brain areas or in precious human samples. Sensitivity of the triple-stage quadrupole mass spectrometer is an important parameter when analyzing low abundance proteins in the experiments described above. Additionally, the MouseExpress® Brain Tissue SILAM standard acts as a carrier, preventing non-specific losses of low abundance proteins in precious samples. Finally, due to the highly conserved nature of neuronal proteins across mammalian species,[1] *MouseExpress® Brain Tissue can be used to quantify synaptic proteins in many animal models, and, perhaps most importantly, human tissue.*

We have successfully utilized the method described above to quantify synaptic proteins in five subcellular fractions prepared from human brain tissue. Currently, the method has been validated for 120 proteins. This number will continue to expand as new data sets are continuously being published and online databases, such as SRM Altas, grow. The addition of SILAM standards prepared from MouseExpress® Brain Tissue to subcellular fractionation-targeted LC-SRM/MS methodologies represents an essential powerful new tool for the study of neuropsychiatric illness.

(continued)



Chromatogram for Synaptotagmin I peptide LTVVILEAK: The non-redundant tryptic peptide LTVVILEAK was monitored in the endogenous "light" form and labeled "heavy" form. Chromatograms represent the signal from three SRM transitions representing the precursor MH+2 ion to the y5, y6 and y7 fragments in vesicular, presynaptic and PSD fractions.

A) Chromatograms for the "light" endogenous peptide monitored by three SRM m/z transitions in vesicular, presynaptic and PSD fractions.

B) Chromatograms for the "heavy" MouseExpress® peptides monitored by three SRM m/z transitions for the same fragment ions in the same fractions.

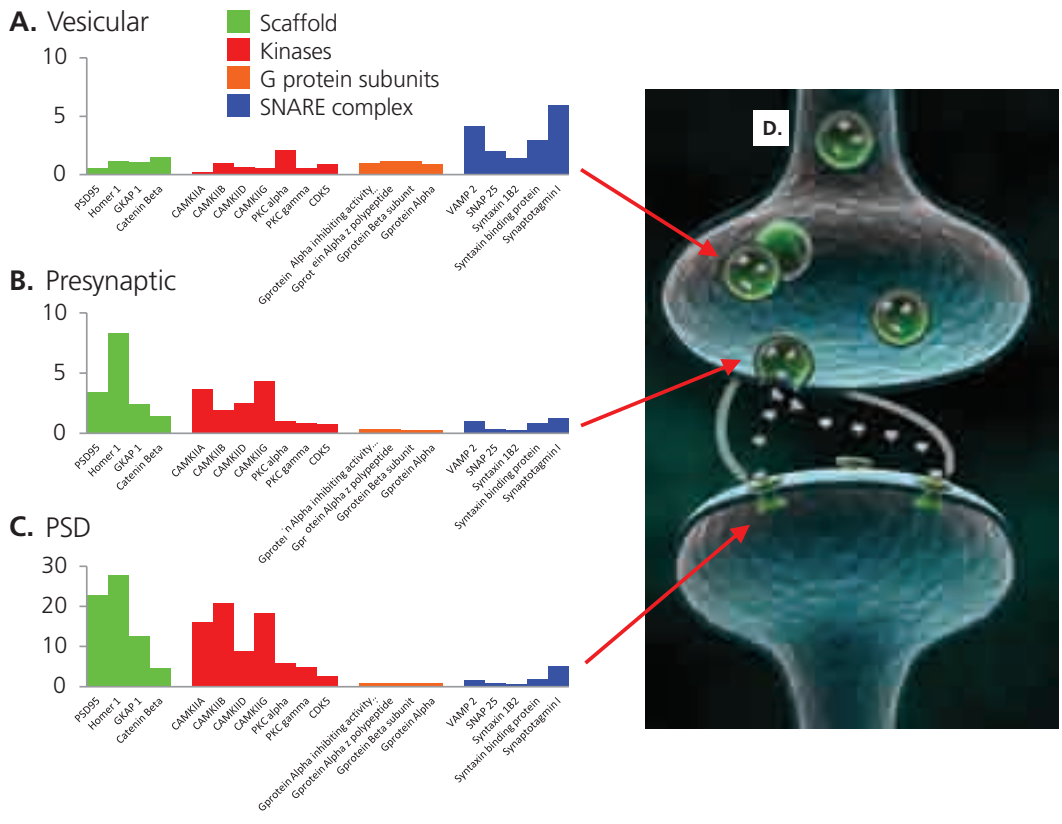


Figure 3. Reciprocal Enrichment of Synaptic Proteins: Relative amounts of select scaffold proteins, kinases, G protein subunits and SNARE complex proteins in vesicular **(A)**, presynaptic **(B)** and PSD **(C)** biochemical fractions are reported. SNARE complex proteins are enriched in the vesicular fraction over other proteins, while scaffold proteins and kinases are moderately enriched in the presynaptic fraction and substantially enriched in the PSD. **(D)** A depiction of the synaptic biochemical fractions.

Related Products

MouseExpress® Mouse Tissue

Catalog No.	Description
MT-LYSC6-MB	MouseExpress® Brain Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FB	MouseExpress® Brain Tissue (F) L-Lysine ($^{13}C_6$, 97%)

MouseExpress® Mouse Feed

Catalog No.	Description
MLK-LYS-C	MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed Kit Kit contains: 1 kg of L-Lysine $^{13}C_6$ feed and 1 kg of (unlabeled) feed

For a complete listing of MouseExpress® Mouse Feed and Tissue, see pages 148-149.

The authors would like to thank Dr. Jonathan C. Trinidad, University of California San Francisco, for helpful suggestions and providing supplemental discovery data for SRM development. We would also like to thank Eksigent, Dublin, CA, for providing a NanoLC-Ultra 2D instrument over a collaborative evaluation period for LC-SRM/MS development.

References

1. Bayés, A.; van de Lagemaat, L.N.; Collins, M.O.; Croning, M.D.; Whittle, I.R.; Choudhary, J.S.; Grant, S.G. **2011**. Characterization of the proteome, diseases and evolution of the human postsynaptic density. *Nat Neurosci, 14*, 19-21.
2. Fernández, E.; Collins, M.O.; Uren, R.T.; Kopanitsa, M.V.; Komiyama, N.H.; Croning, M.D.; Zografos, L.; Armstrong, J.D.; Choudhary, J.S.; Grant, S.G. **2009**. Targeted tandem affinity purification of PSD-95 recovers core postsynaptic complexes and schizophrenia susceptibility proteins. *Mol Syst Biol, 5*, 269.
3. Chaerkady, R.; Pandey, A. **2007**. Quantitative proteomics for identification of cancer biomarkers. *Proteomics Clin Appl, 1*, 1080-9.
4. Trinidad, J.C.; Thalhammer, A.; Specht, C.G.; Lynn, A.J.; Baker, P.R.; Schoepfer, R.; Burlingame, A.L. **2008**. Quantitative analysis of synaptic phosphorylation and protein expression. *Mol Cell Proteomics*, *7*, 684-96.
5. Reissner, K.J.; Uys, J.D.; Schwacke, J.H.; Comte-Walters, S.; Rutherford-Bethard, J.L.; Dunn, T.E.; Blumer, J.B.; Schey, K.L.; Kalivas, P.W. **2011**. AKAP Signaling in Reinstated Cocaine Seeking Revealed by iTRAQ Proteomic Analysis. *J Neurosci, 31*, 5648-58.
6. Hahn C.G.; Banerjee, A.; Macdonald, M.L.; Cho, D.S.; Kamins, J.; Nie, Z.; Borgmann-Winter, K.E.; Grosser, T.; Pizarro. A.; Ciccimaro, E.; Arnold, S.E.; Wang, H.Y.; Blair, I.A. **2009**. The post-synaptic density of human postmortem brain tissues: an experimental study paradigm for neuropsychiatric illnesses. *PLoS One, 4*, e5251.

Synthetic Peptides As Internal Standards

Dr. Catherine Fenselau
University of Maryland, College Park, MD 20742 USA

The addition of synthetic internal standards has been used for nearly 40 years for quantitation with mass spectrometry.[1] It is particularly useful with LC-ES-MS and MALDI-MS for proteomic analyses. In the most common workflow for proteomics, peptides are selected based on uniqueness, stability, chromatographic behavior and sensitivity to ionization techniques to serve as surrogate markers for proteins of interest. Internal standards are added to allow these peptides and the proteins they represent to be quantified. The best internal standards are peptides that have identical sequences as the biomarker peptides, and carry stable isotopic labels.[2] The isotopes change the mass, but not the chemical behavior. Isotope ratios of spiked peptides and peptides obtained from protein biomarkers are measured by mass spectrometry and tandem mass spectrometry to provide absolute quantitation of the endogenous protein.[3,4] The use of internal standards allows multiple peptides (and thus multiple proteins) to be quantified in a single sample, and facile quantitation of a particular peptide in multiple samples, *e.g.,* at multiple time points. The approach is applicable to quantitation of proteins in tissue samples as well as proteins in solution. Peptides with homologous sequences and different masses (and no isotope labels) can also be used as internal standards, supported by standard curves. Isotope-labeled proteins provide even better internal standards because they undergo fractionation and digestion along with the biomarker proteins and provide correction for losses that occur before peptides are produced. Such proteins may be provided from normal or recombinant cells grown in labeled media.

References

1. Fenselau, C. **1977**. The mass spectrometer as a gas chromatograph detector. *Anal Chem, 49,* 563A-570A.
2. Desiderio, D.M.; Kai, M. **1983**. Preparation of stable isotope-incorporated peptide internal standards for field desorption mass spectrometry quantification of peptides in biologic tissue. *Biomed Mass Spectrom, 10*, 471-479.
3. Gerber, S.A.; Rush, J.; Stemman, O.; Kirschner, M.W.; Gygi, S.P. **2003**. Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. *Proc Natl Acad Sci USA, 100*, 6940-6945.
4. Anderson, L.; Hunter, C.L. **2006**. Quantitative mass spectrometric multiple reaction monitoring assays for major plasma proteins. *Mol Cell Proteomics, 5*, 573-588.

"The commercial availability of stable isotope-labeled amino acids with very high isotopic purity has revolutionized quantitative proteomics. From their use in metabolic labeling of cells and rodents for differential discovery proteomics, to their use in synthetic peptides as internal standards for targeted analysis of proteins, isotopically labeled amino acids make it possible to measure, with very high precision, changes in the levels of peptides and the proteins they are derived from in highly complex samples such as cell lysates, tissue and plasma. Cambridge Isotope Labs has been and continues to be a leader in the commercial production of labeled amino acids and other labeled compounds."

Dr. Steven A. Carr
Broad Institute of MIT and Harvard

Protected Amino Acids

Stable isotope-labeled peptides are used in biomarker discovery and validation, as well as in drug and metabolite monitoring, peptide signaling experiments, metabolomics and pharmacokinetics. CIL is pleased to offer many of these uniformly labeled protected amino acids at 99% isotopic purity for use in the synthesis of peptides. Please see the complete listing of protected amino acids for peptide synthesis on pages 135-136.

99% Enriched Protected Amino Acids

Catalog No.	Description
CNLM-4355-H	L-Alanine-*N*-FMOC ($^{13}C_3$, 99%; ^{15}N, 99%)
CLM-8475-H	L-Arginine-*N*-FMOC, Pbf ($^{13}C_6$, 99%) (contains solvent)
CNLM-8474-H	L-Arginine-*N*-FMOC, Pbf ($^{13}C_6$, 99%; $^{15}N_4$, 99%) (contains solvent)
CNLM-6193-H	L-Asparagine-*N*-FMOC, β-*N*-trityl ($^{13}C_4$, 99%; $^{15}N_2$, 99%)
CNLM-4752-H	L-Aspartic acid-*N*-FMOC, β-*O*-*t*-butyl ester ($^{13}C_4$, 99%; ^{15}N, 99%)
CNLM-4722-H	L-Cysteine-*N*-FMOC, *S*-trityl ($^{13}C_3$, 99%; ^{15}N, 99%)
CNLM-4356-H	L-Glutamine-*N*-FMOC ($^{13}C_5$, 99%; $^{15}N_2$, 99%)
CNLM-7252-H	L-Glutamine-*N*-FMOC, γ-*N*-trityl ($^{13}C_5$, 99%; $^{15}N_2$, 99%)
CNLM-4753-H	L-Glutamic acid-*N*-FMOC, γ-*t*-butyl ester ($^{13}C_5$, 99%; ^{15}N, 99%) CP 96%
CNLM-4357-H	Glycine-*N*-FMOC ($^{13}C_2$, 99%; ^{15}N, 99%)
CNLM-4346-H	L-Isoleucine-*N*-FMOC ($^{13}C_6$, 99%; ^{15}N, 99%)
CNLM-4345-H	L-Leucine-*N*-FMOC ($^{13}C_6$, 99%; ^{15}N, 99%)
CLM-7865-H	L-Lysine-α-*N*-FMOC, ε-*N*-*t*-BOC ($^{13}C_6$, 99%)
CNLM-4754-H	L-Lysine-α-*N*-FMOC, ε-*N*-*t*-BOC ($^{13}C_6$, 99%; $^{15}N_2$, 99%)
CNLM-4358-H	L-Methionine-*N*-FMOC ($^{13}C_5$, 99%; ^{15}N, 99%)
CNLM-4362-H	L-Phenylalanine-*N*-FMOC ($^{13}C_9$, 99%; ^{15}N, 99%)
CNLM-4347-H	L-Proline-*N*-FMOC ($^{13}C_5$, 99%; ^{15}N, 99%)
CNLM-8403-H	L-Serine-*N*-FMOC ($^{13}C_3$, 99%; ^{15}N, 99%)
CNLM-4755-H	L-Serine-*N*-FMOC, *O*-*t*-butyl ether ($^{13}C_3$, 99%; ^{15}N, 99%)
CNLM-7615-H	L-Threonine-*N*-FMOC, *O*-*t*-butyl ether ($^{13}C_4$, 99%; ^{15}N, 99%)
CNLM-4349-H	L-Tyrosine-*N*-FMOC, *O*-*t*-butyl ether ($^{13}C_9$, 99%; ^{15}N, 99%)
CNLM-4348-H	L-Valine-*N*-FMOC ($^{13}C_5$, 99%; ^{15}N, 99%)

CIL supplies these highly enriched amino acids to the world's leading peptide manufacturers.

"I have been dealing with Cambridge Isotope Labs for over a decade, and simply put, CIL is the best isotope company out there – best in quality, best in service..."

Samuel Massoni, CEO
New England Peptide, Inc.

"For many years we have had a strong relationship with CIL. The Heavy Peptides AQUA standards we produce require the highest quality of raw material, service and technical support, and CIL never failed to deliver. When we asked CIL to adapt the specifications of their products so we could meet the needs of our customers, they reacted immediately and within a couple of months they were ready to deliver."

Joel Louette, MS
Commercial Director
Thermo Scientific Custom Biopolymers

"With their combination of high quality, extensive catalogue and excellent customer relations, CIL is always our first port of call when sourcing isotopically labelled protected amino acid derivatives. "

Dr. Iain Pritchard
Assistant Director of Production
Bachem UK Ltd., St. Helens, UK

Preloaded Resins

NEW Synthesis-Ready Preloaded Resins for Solid-Phase Synthesis of Stable Isotope-Labeled Tryptic Peptides

These 2-ClTrt-resins, originally introduced by Barlos and coworkers,[1] are ideal for the synthesis of isotopically labeled tryptic peptides for stable isotope dilution (SID)/selective reaction monitoring (SRM) assays for quantitative proteomics. The resins are prepared from labeled, protected amino acids with the highest chemical, isotopic and chiral purity available. Additionally, because enantiomerization does not occur during the loading of 2-ClTrt resin,[2] peptides containing C-terminal-labeled-arginine (Pbf) or labeled-lysine (BOC) residues can be produced essentially free from contamination by diastereomeric side-products.

2-ClTrt resins are excellent supports for use in standard solid-phase peptide synthesis protocols. Final cleavage of the peptides from the resin with standard trifluoroacetic acid-based cocktails leads to fully deprotected peptide C-terminal acids terminated with an isotopically labeled arginine (Δm = +10 amu) or lysine (Δm = +8 amu). Alternative mild acid protocols[3] can provide isotopically labeled, protected peptide fragments.

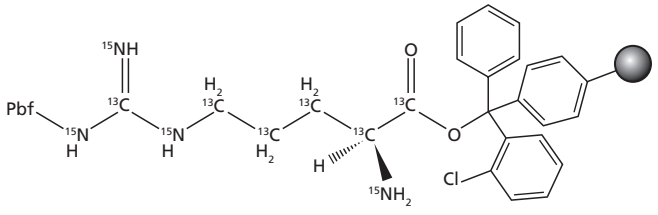
Product Description

The resin is prepared from the highest quality cross-linked polystyrene (1% divinylbenzene) with the 2-chlorotrityl linkage directly attached to the support.

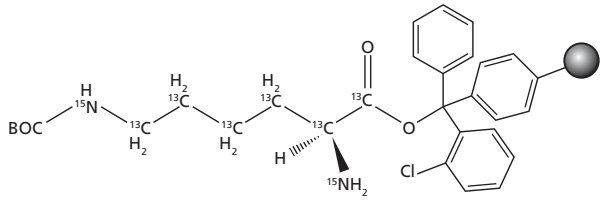
Loading: 0.25-0.5 mmol/g; copoly (styrene-1% DVB)
Particle size: 100-200 mesh
Reagents: L-Arginine-*N*-FMOC, Pbf ($^{13}C_6$, 99%; $^{15}N_4$, 99%)
or
L-Lysine-α-*N*-FMOC, ε-*N*-*t*-BOC ($^{13}C_6$, 99%; $^{15}N_2$, 99%)
Isotope Enrichment: >99%
Chiral Purity: >99%

Catalog No.	Description
SRPR-ARG-CN	L-Arginine (Pbf) ($^{13}C_6$, 99%; $^{15}N_4$, 99%) – 2-ClTrt resin
SRPR-LYS-CN	L-Lysine (BOC) ($^{13}C_6$, 99%; $^{15}N_2$, 99%) – 2-ClTrt resin

SRPR-ARG-CN L-Arginine (Pbf) ($^{13}C_6$, 99%; $^{15}N_4$, 99%) – 2-ClTrt resin



SRPR-LYS-CN L-Lysine (BOC) ($^{13}C_6$, 99%; $^{15}N_2$, 99%) – 2-ClTrt resin



References

1. Barlos, K.; Gatos, D.; Kallitsis, J.; Papaphotiu, G.; Sotiriu, P.; Yao, W.Q.; Schafer, W. **1989**. Synthesis of Protected Peptide-Fragments Using Substituted Triphenylmethyl Resins. *Tetrahedron Lett, 30*, 3943-3946.
2. Fujiwara, Y.; Akaji, K.,; Kiso, Y. **1994**. Racemization-Free Synthesis of C-Terminal Cysteine-Peptide Using 2-Chlorotrityl Resin. *Chem Pharm Bull, 42*, 724-726.
3. Barlos, K.; Gatos. D. **1999**. 9-Fluorenylmethyloxycarbonyl/tButyl-based convergent protein synthesis. *Biopolymers, 51*, 266-278.

Chemical Tagging Reagents and Related Products

To fully understand the function of the proteome in health and disease, one must have the ability to accurately quantify protein in many different types of biological samples. The accuracy of mass spectrometric quantitative proteomic measurements is improved using heavy isotope-enriched internal standards. The most commonly used isotope-enriched internal standards are a single cell line, a mixture of cell lines, or tissue. Metabolic incorporation of heavy isotopes into a proteome, such as in SILAC or SILAM, is the preferred method to prepare internal standards, however, many organisms and animals are not amenable to metabolic incorporation. Fortunately, proteins or peptides may be easily modified through chemical synthesis using relatively simple chemical or "tagging" reagents. Tagging reagents are compatible with almost any type of biological sample type and often represent a low-cost alternative to metabolic labeling. CIL carries a full line of labeled tagging reagents so that the incorporation of stable isotopes at either the peptide or protein level is easily achieved.

Dimethylation

Many sample types are not amenable to metabolic incorporation. Fortunately, proteins in essentially any type of biological sample can be modified by reductive methylation. Reductive methylation utilizes formaldehyde and cyanoborohydride or cyanoboro-deuteride and results in the addition of two methyl groups on the N-terminus and lysine side chains. CIL offers various forms of labeled formaldehyde and cyanoborodeuteride for the rapid labeling of peptides. Dimethylation tagging method is thus universal in that it is compatible with essentially any protein sample and represents a low-cost alternative to metabolic labeling. Dimethylation allows for both duplex and triplex modes of operation.

Related Products

Catalog No.	Description
CLM-806	Formaldehyde (^{13}C, 99%) (~20% w/w in H_2O)
DLM-805	Formaldehyde (D_2, 98%) (~20% w/w in D_2O)
CDLM-4599	Formaldehyde (^{13}C, 99%; D_2, 98%) (20% w/w in D_2O)
DLM-7364	Sodium cyanoborodeuteride (D_3, 98%)

"Proteome Sciences has developed and manufactures the isobaric Tandem Mass Tag® (TMT®) reagents, which are sold under license by Thermo Fisher Scientific. TMT® reagents for MS-based proteomics investigations are used worldwide and represent one of our key business activities. To meet increased market needs and to make full advantage of latest MS instrument developments, we continuously evaluate improvement potential together with our partners and key opinion leaders. Heavy isotope-labeled building blocks are key to both guarantee a constant TMT® product supply to our licensee and to generate new prototypes and TMT® products. Since we started manufacturing TMT®'s in large scale in 2008, Cambridge Isotope Labs has consistently been a reliable partner who can provide us with bulk amounts of established precursors and supply custom-tailored precursors for improvement investigation. Their product quality both in chemical purity, isotope enrichment and delivery times have been a sustainable source for our business. We are looking forward to continuing our collaboration with CIL as we develop exciting new tags for enhanced mass spectrometry methods."

Dr. Karsten Kuhn
Head of Chemistry
Proteome Sciences, Frankfurt, Germany

Tandem Mass Tag® (TMT®) are registered trademarks of Proteome Sciences

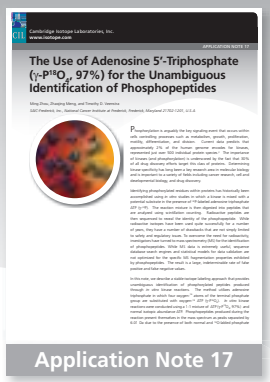
"We have come to depend on CIL to furnish us with high quality reagents during the past few years as our laboratory became interested in the synthesis of a series of isotopically labeled coding agents for quantification in both proteomics and metabolomics. CIL was both knowledgeable and helpful, being willing to listen to our problems and going to their technical staff for solutions. Relationships like this are infrequent today and extremely valuable when you need answers quickly."

Dr. Fred E. Regnier
J.H. Law Distinguished Professor
Department of Chemistry, Purdue University

Chemical Tagging Reagents and Related Products

Catalog No.	Description
CLM-173	Acetaldehyde (1,2-$^{13}C_2$, 99%)
DLM-112	Acetaldehyde (D_4, 99%)
CLM-1159	Acetic anhydride (1,1′-$^{13}C_2$, 99%)
CLM-1160	Acetic anhydride (2,2′-$^{13}C_2$, 99%)
CLM-1161	Acetic anhydride (1,1′,2,2′-$^{13}C_4$, 99%)
DLM-1162	Acetic anhydride (D_6, 98%)
CDLM-9271	Acetic anhydride ($^{13}C_4$, 99%; D_6, 98%)
DLM-9	Acetone (D_6, 99.9%)
CLM-1260	Acetonitrile (1-^{13}C, 99%)
CLM-704	Acetyl chloride (1,2-$^{13}C_2$, 99%)
DLM-247	Acetyl chloride (D_3, 98%)
CDLM-6208	Acetyl chloride ($^{13}C_2$, 99%; D_3, 98%)
CLM-9270	Acrylamide (1-^{13}C, 99%)
CLM-813	Acrylamide (1,2,3-$^{13}C_3$, 99%)
DLM-821	Acrylamide (2,3,3-D_3, 98%)
OLM-7858	Adenosine 5′-triphosphate, sodium salt (γ-$^{18}O_4$, 94%+)
CLM-8906	*S*-Adenosyl-L-homocysteine (adenosine-$^{13}C_{10}$, 98%)
CLM-8755	β-Alanine (3-^{13}C, 99%)
CLM-8756	β-Alanine (1,2,3-$^{13}C_3$, 99%)
CNLM-3440	β-Alanine (3-^{13}C, 99%; ^{15}N, 98%)
CNLM-3946	β-Alanine (U-$^{13}C_3$, 98%+; ^{15}N, 96-99%)
CLM-714	Aniline ($^{13}C_6$, 99%)
CLM-466	Barium carbonate (^{13}C, 98%+)
CLM-182	Benzene ($^{13}C_6$, 99%)
CLM-1813	Benzoic acid (ring-$^{13}C_6$, 99%)
CLM-3010	Benzoyl chloride (carbonyl-^{13}C, 99%)
DLM-595	Benzoyl chloride (D_5, 99%)
CLM-1339	Bromoacetic acid (1,2-$^{13}C_2$, 99%)
CLM-871	Bromobenzene ($^{13}C_6$, 99%)
DLM-398	Bromobenzene (D_5, 99%)
DLM-103	2-Bromoethanol (1,1,2,2-D_4, 98%) CP ≥95%
CLM-1829	Chlorobenzene ($^{13}C_6$, 99%)
DLM-341	1,4-Dibromobenzene (D_4, 98%)
CDLM-301	1,2-Dibromoethane (1,2-$^{13}C_2$, 99%; D_4, 98%)
CLM-495	Diethyl malonate (2-^{13}C, 99%)
CLM-3603	Diethyl malonate (1,2,3-$^{13}C_3$, 99%)
DLM-267	Dimethylamine (D_6, 99%) gas
CLM-266	Dimethyl sulfate ($^{13}C_2$, 99%)
DLM-196	Dimethyl sulfate (D_6, 98%)
DLM-2622	DL-1,4-Dithiothreitol (D_{10}, 98%)
DLM-6785	1,2-Ethanedithiol (1,1,2,2-D_4, 98%)
DLM-552	Ethanolamine (D_4, 98%)
CLM-3297	Ethyl acetoacetate (1,2,3,4-$^{13}C_4$, 99%)
CLM-1009	Ethyl bromoacetate (1-^{13}C, 99%)
CLM-1011	Ethyl bromoacetate (1,2-$^{13}C_2$, 99%)
DLM-271	Ethylene oxide (D_4, 98%) (stabilized with 0.1% hydroquinone)

Catalog No.	Description
DLM-6711	*N*-Ethylmaleimide (ethyl-D_5, 98%)
CLM-806	Formaldehyde (^{13}C, 99%) (~20% w/w in H_2O)
DLM-805	Formaldehyde (D_2, 98%) (~20% w/w in D_2O)
CDLM-4599	Formaldehyde (^{13}C, 99%; D_2, 98%) (20% w/w in D_2O)
DLM-1229	Glycerol (1,1,2,3,3-D_5, 99%)
CNLM-7138	Guanidine•HCl (^{13}C, 99%; $^{15}N_3$, 98%)
CNLM-7333	Guanidine•HBr (^{13}C, 99%: $^{15}N_3$, 98%)
DLM-7249	Iodoacetamide (D_4, 98%)
CLM-3264	Iodoacetic acid (2-^{13}C, 99%)
CLM-8824	Iodoacetic acid ($^{13}C_2$, 99%)
DLM-272	Iodoethane (D_5, 99%) + copper wire
DLM-1136	Isopropanol (dimethyl-D_6, 98%)
DLM-1981	Methanesulfonic acid (D_4, 97-98%)
DLM-598	Methanol (D_3, 99.5%)
CDLM-688	Methanol (^{13}C, 99%; D_4, 99%)
CDLM-8241	Methylamine•HCl (^{13}C, 99%; methyl-D_3, 98%)
CDNLM-8182	Methylamine•HCl (^{13}C, 99%; methyl-D_3, 98%; ^{15}N, 98%)
CNLM-6088	*O*-Methylisourea hydrogen chloride (isourea-^{13}C, 99%; $^{15}N_2$, 98%) CP ≥95%
DLM-2872	Nicotinic acid, ethyl ester (2,4,5,6-D_4, 98%)
CLM-675	Nitrobenzene ($^{13}C_6$, 99%)
CLM-6586	2-Nitrobenzenesulfenyl chloride ($^{13}C_6$, 99%)
CLM-216	Phenol ($^{13}C_6$, 99%)
DLM-7731	Phenyl isocyanate (phenyl-D_5, 98%)
OLM-1057	Phosphoric acid ($^{18}O_4$, 96%) (80-85% in ^{18}O water)
NLM-111	Potassium cyanide (^{15}N, 98%+)
OLM-7493	Potassium dihydrogen phosphate ($^{18}O_4$, 97%)
OLM-7523	Potassium phosphate (^{18}O, 97%)
DLM-599	Propionic acid (D_6, 98%)
DLM-3305	Propionic anhydride (D_{10}, 98%)
DLM-1067	1,2-Propylene oxide (D_6, 98%) (stabilized with hydroquinone)
DLM-3126	Sodium acetate (D_3, 99%)
CDLM-611	Sodium acetate (1-^{13}C, 99%; D_3, 98%)
CDLM-1240	Sodium acetate (2-^{13}C, 99%; D_3, 98%)
CDLM-3457	Sodium acetate (1,2-$^{13}C_2$, 99%; D_3, 98%)
DLM-226	Sodium borodeuteride (D_4, 99%)
DLM-7364	Sodium cyanoborodeuteride (D_3, 98%)
CLM-1571	Succinic acid ($^{13}C_4$, 99%)
CDLM-7754	Succinic acid ($^{13}C_4$, 99%; 2,2,3,3-D_4, 98%)
CLM-2473	Succinic anhydride (1,2,3,4-$^{13}C_4$, 99%)
DLM-833	Succinic anhydride (D_4, 98%)
DLM-6143	Suberic acid (2,2,7,7-D_4, 98%)
DLM-1176	Toluene (ring-D_5, 98%)
CLM-311	Urea (^{13}C, 99%)
NLM-233	Urea ($^{15}N_2$, 98%)



The Use of Adenosine 5′-Triphosphate (γ-$P^{18}O_4$, 97%) for the Unambiguous Identification of Phosphopeptides

Ming Zhou, Zhaojing Meng, and Timothy D. Veenstra
SAIC-Frederick, Inc., National Cancer Institute at Frederick
Frederick, MD 21702-1201 USA

Phosphorylation is arguably the key signaling event that occurs within cells controlling processes such as metabolism, growth, proliferation, motility, differentiation and division. Current data predicts that approximately 2% of the human genome encodes for kinases, represented just over 500 individual protein species.[2] The importance of kinases (and phosphorylation) is underscored by the fact that 30% of all drug discovery efforts target this class of proteins. Determining kinase specificity has long been a key research area in molecular biology and is important to a variety of fields including cancer research, cell and developmental biology and drug discovery.

Identifying phosphorylated residues within proteins has historically been accomplished using *in vitro* studies in which a kinase is mixed with a potential substrate in the presence of ^{32}P-labeled adenosine triphosphate ATP (γ-^{32}P). The reaction mixture is then digested into peptides that are analyzed using scintillation counting. Radioactive peptides are then sequenced to reveal the identity of the phosphopeptide. While radioactive isotopes have been used quite successfully for a number of years, they have a number of drawbacks that are not simply limited to safety and regulatory issues. To overcome the need for radioactivity, investigators have turned to mass spectrometry (MS) for the identification of phosphopeptides. While MS data is extremely useful, sequence database search engines and statistical models for data validation are not optimized for the specific MS fragmentation properties exhibited by phosphopeptides. The result is a large, indeterminable rate of false positive and false negative values.

In this note, we describe a stable isotope-labeling approach that provides unambiguous identification of phosphorylated peptides produced through *in vitro* kinase reactions. The method utilizes adenosine triphosphate in which four oxygen-16 atoms of the terminal phosphate group are substituted with Adenosine 5′-triphosphate, sodium salt (γ-$^{18}O_4$, 97%) (OLM-7858). *In vitro* kinase reactions were conducted using a 1:1 mixture of ATP (γ-$P^{18}O_4$, 97%) and normal isotopic abundance ATP. Phosphopeptides produced during the reaction present themselves in the mass spectrum as peaks separated by 6.01 Da due to the presence of both normal and ^{18}O-labeled phosphate groups.

Results

In vitro phosphorylation using ATP (γ-$P^{18}O_4$)

The utility of Adenosine 5′-triphosphate, sodium salt (γ-$^{18}O_4$, 97%) as a novel reagent for identifying phosphopeptides was evaluated in a series of reactions using a known kinase/substrate *in vitro* reaction. Myelin basic protein (MBP) was phosphorylated *in vitro* using MAP kinase in the presence of a 1:1 mixture of normal isotopic abundance ATP and ATP (γ-$P^{18}O_4$, 97%). The mass spectrum of the reaction products is shown in Figure 1. The MALDI-TOF/MS spectrum is dominated by singlet peaks, however, a doublet of signals at mlz ratios 1571.79 and 1577.81 (Δm/z = 6.02) was observed. The accurate mass, as well as the tandem MS data (not shown), confirmed the doublet as $^{16}O_3$PO- and $^{18}O_3$PO-labeled versions of the peptide NIVpTPRTPPPSQGK. Site-specific assignment of the phosphothreonine residue was determined by the tandem MS data.

A triplet of signals at m/z ratios 1651.76, 1657.77 and 1663.79 was also observed in the mass spectrum shown in Figure 1. The accurate mass and tandem MS data identified these signals as originating from the doubly phosphorylated peptide, NIVpTPRpTPPPSQGK. The peak at m/z 1651.76 corresponded to the phosphopeptide containing two light-isotopic (*i.e.* $^{16}O_3$PO-) phosphate groups. The peak at m/z 1663.79 corresponded to the peptide containing two heavy-isotopic (*i.e.* $^{18}O_3$PO-) phosphate groups. The middle peak at 1657.77 was identified as the phosphopeptide containing both a light and heavy isotopic phosphate group. The phosphate modifications to Thr94 and Thr97 of MBP were readily assigned using the tandem MS data.

Evaluation of Isotope Exchange Loss

Depending on the type and structural position of the isotope, exchange loss can be a concern when utilizing stable isotope labeling. While no obvious reason for a natural exchange loss of ^{18}O atoms of ATP (γ-$P^{18}O_4$) for ^{16}O when dissolved in H_2O was clear, the effect, if any, of the kinase activity on exchange of the phosphate oxygen atoms was not known. Therefore, a second series of *in vitro* kinase reactions were performed in which a synthetic peptide (KVEKIGEGTYGVVYK), representing residues 6-20 of Cdc2 was incubated with the tyrosine kinase Src in the

(continued)

presence of ATP (γ-$P^{18}O_4$). The reaction was performed in both native H_2O (*i.e.* $H_2{}^{16}O$) and $H_2{}^{18}O$. As shown in Figure 2, the MALDI-TOF/MS spectra of the phosphopeptides were identical regardless of whether the kinase reactions are run in $H_2{}^{16}O$ or $H_2{}^{18}O$ showing that no isotope exchange loss occurs.

Discussion

Adenosine 5′-Triphosphate, sodium salt (γ-$^{18}O_4$, 97%) represents a new, non-radioactive, proteomic reagent for identifying phosphopeptides using MS. Other labeling methods either chemically modify the phosphorylation site, usually by removing the phosphate group first and then modifying the reactive site that is left behind with a tag. This method is the first that simply incorporates a stable isotope label on the native phosphate. The data shows the first example of a stable isotope-labeling method for phosphopeptides in which the label is part of the phosphate group. Conducting *in vitro* kinase reactions using a 1:1 mixture of ATP and ATP (γ-$P^{18}O_4$, 97%) produces a distinct peak signature within the mass spectrum, providing absolute confidence in the phosphorylation status of the peptide. This distinctive signature represents a tremendous benefit to peptide mapping experiments that rely solely on accurate mass measurement for phosphopeptide identification.

The use of a non-radioactive phosphate tag for the identification of phosphorylation sites eliminates a number of problems associated with ATP (γ-^{32}P). Besides all of the associated health issues and precautions that must be taken, experiments using ATP (γ-^{32}P) require extremely careful planning and timing so that the precise amount needed for a specific experiment is ordered and consumed within a relatively short period of time upon delivery. After completion of the experiment, all of the unused reagent and any consumable materials that it came in contact with must be properly disposed. In comparison to its radioactive counterpart, ATP (γ-$P^{18}O_4$, 97%) shelf-life is comparable to normal ATP and requires no special precautions or disposal procedures.

The identification of kinase-induced phosphorylation sites in target proteins is critical for the understanding of the biological processes mediated by these kinases. The technique described here is a novel, non-radioactive method that enables phosphorylation sites identification. Samples can be safely manipulated without the need for radioactive tags and the need for other precautions needed when working with ^{32}P. This reagent will have a positive impact by decreasing the false positive rate associated with the identification of phosphopeptides using MS.

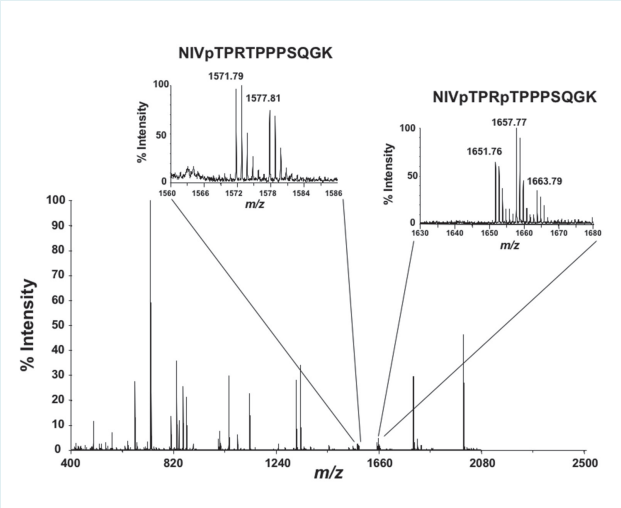


Figure 1. Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry spectra of *in vitro* kinase reaction products of MAP kinase and myelin basic protein in the presence of a 1:1 mixture of ATP (γ-$P^{18}O_4$). **A)** Mono- and di-phosphorylated versions of the tryptic peptide NIVTPRTPPPSQGK were observed in the mass spectrum (insets).

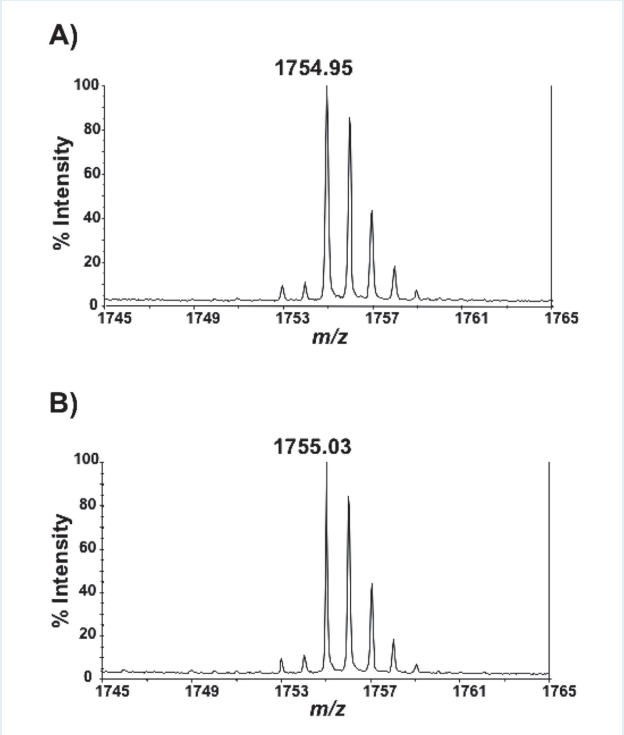


Figure 2. Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry spectra of the synthetic peptide (KVEKIGEGTYGVVYK) phosphorylated by Src protein tyrosine kinase using ATP (γ-$P^{18}O_4$) in **A)** H_2O and **B)** $H_2{}^{18}O$.

Acknowledgements

This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract NO1-CO-12400. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organization imply endorsement by the United States Government. This was presented as an oral presentation at the 2007 American Society of Mass Spectrometry, Indianapolis 2007.

Competing Interests Statement

The authors declare no competing financial interests.

Related Product

Catalog No.	Description
OLM-7858	Adenosine 5'-triphosphate, sodium salt (γ-$^{18}O_4$, 94%+)

References

Zhou, M.; Meng, Z.; Jobson, A.G.; Pommier, Y.; Veenstra, T.D. **2007**. Detection of Protein Phosphorylation Sites using γ [$^{18}O_4$]-ATP and Mass Spectrometry. Submitted for publication.

Heller, M. **2003**. Trypsin catalyzed ^{16}O-to-^{18}O exchange for proteomics. *J Am Soc Mass Spectrom, 14*, 704.

Yao, X.; Freas, A.; Ramirez, J.; Demirev, P.; Fenselau, C. **2001**. Proteolytic ^{18}O Labeling for Comparative Proteomics: Model Studies with Two Serotypes of Adenovirus. *Anal Chem, 73*, 2836-2842.

Reynolds, K.; Yao, X.; Fenselau, C. **2002**. Mass Spectrometric Analysis of Glu-C Catalyzed Global ^{18}O Labeling for Comparative Proteomics. *J Proteome Research*, *1,* 27-33.

Berhane, B.T.; Limbach, P.A. **2003**. Stable isotope labeling for matrix-assisted laser desorption/ionization mass spectrometry and post-source decay analysis of ribonucleic acids. *J Mass Spectrom, 38*, 872-8.

Rich *E. coli* Media

Stable isotope-labeled cellular biomass can be used in both proteomic and metabolomic investigations. In addition, quantitative proteomic MS-based studies can benefit greatly from the use of purified, labeled intact protein as internal standards. The use of properly folded, labeled intact proteins are ideal internal standards because they mimic the physical and chemical properties of the target endogenous protein in a sample prior to, during and after digestion. In particular, they undergo a similar degree of proteolytic cleavage as the unlabeled counterpart, thus improving the accuracy of the IDMS experimental result for both middle-down or bottom-up methodologies.

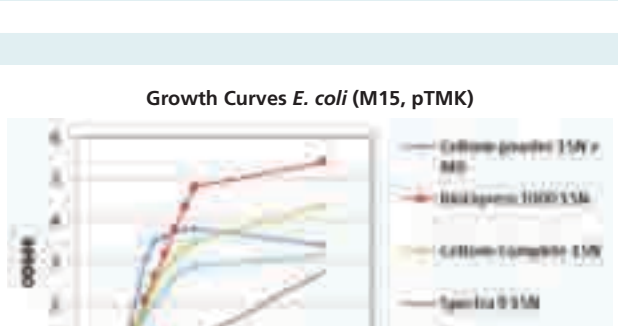
BioExpress® 1000

BioExpress® 1000 is CIL's all-time classic rich bacterial cell growth medium. BioExpress® 1000 provides excellent growth and expression characteristics for a number of different bacterial systems. BioExpress® 1000 contains nearly the same level of amino acids as LB medium. Glucose levels range from 0.1-0.5 g/L, depending on the batch. BioExpress® 1000 media is prepared by adding sterile cell culture-grade water and mixing. Please note that D_2O is required for reconstitution for products containing deuterium. BioExpress® 1000 is supplied as a 100 mL sterile liquid 10x concentrate, and reconstitutes to 1 L with no final pH adjustment required; 10 mL sample sizes are also available. The 10 mL sample size reconstitutes to make 100 mL of media with no final pH adjustment required.

Please see page 139 for a complete listing of BioExpress® 1000 products.

Growth Curves *E. coli* (BL21, pGFP)

Growth Curves *E. coli* (M15, pTMK)



"In our hands, CIL's BioExpress® 1000 worked like a charm. The cell growth rate and protein expression level essentially matched the results obtained with Luria broth, and the ^{15}N labeling efficiency was excellent."

Tero Pihlajamaa, PhD
Finnish Biological NMR Center
Institute of Biotechnology
University of Helsinki, Finland

Stable isotope-labeled intact proteins

Please contact CIL for additional information.

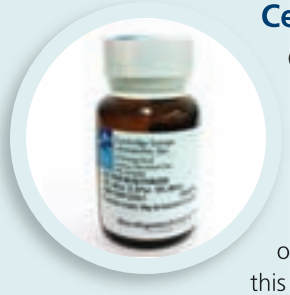
Rich *E. coli* Media

Although growth in minimal media is economical, there is no substitute for the enhanced growth rates and increased levels of protein expression that may be gained by the use of a rich medium. Rich bacterial media are complex formulations that are usually derived from algal hydrolysates and contain all the necessary nutrients to promote excellent growth. CIL offers a number of rich media used in labeled protein expression using bacterial systems.

For pointers on how to maximize protein yield using CIL's BioExpress® 1000 media, please see CIL Application Note 15 at www.isotope.com. Please see CIL Application Note 12 to learn how spiking BioExpress® 1000 media into minimal media provides a low-cost means to enhance the performance of minimal media.

Celtone® Complete

Celtone® Complete yields a growth rate comparable to LB media, allowing for inoculation and induction within one working day. Glucose levels range from ~0.3 to 0.6 g/L, depending on the batch. Celtone® Complete is a ready-to-use medium that does not require dilution or pH adjustment. Each lot is tested for sterility, cell growth and protein expression. Celtone® Complete is available in 0.1 L and 1 L sizes. **Please see page 139 for a complete listing of Celtone® Complete products.**



Celtone® Powder

Celtone® Powder is CIL's most flexible nutrient-rich media. The advantage of Celtone® Powder is that researchers can formulate a custom medium based on their specific research needs. Depending on cell line and desired performance, this powdered media can be used at concentrations ranging from 1 g to 10 g per liter. Truly exceptional performance has been achieved using 10 g of Celtone® Powder in 1 L of medium containing M9 salts, 2-3 g/L of glucose and 1 g of ammonium chloride (see graph on page 52). Because it is a powder, this product has the longest shelf life of any fully rich bacterial cell growth medium. Please note that if deuterium labeling is desired, D_2O must be used in media preparation. Also note that it is normal to have insoluble material present after dissolution. This material may be removed using filter paper prior to sterile filtration and will not affect performance of the medium. Celtone® Powder is available in 0.5 g and 1 g packaged sizes. **Please see page 139 for a complete listing of Celtone® Powder products.**

Spectra 9 Media

Spectra 9 Media is not a fully rich medium, however, it represents a cost-effective medium for *E. coli* growth and protein expression. It is comprised of labeled salts, labeled carbohydrates (>2 g glucose/L), and is supplemented with Celtone® Powder at a concentration of 1 g/L. **Please see page 139 for a complete listing of Spectra 9 Media products.**

Yeast Media and Reagents

The overexpression of protein in yeast cells represents a powerful expression system for the source of properly folded and functional eukaryotic protein. Much of our understanding of biological processes and human diseases can be attributed to studies on model organisms such as yeast. Thus, yeast has been and will continue to be an important model organism for systems biology and for assessing new and existing MS-based proteomic methodologies. The utility of yeast lies in its simple genome, its ease of manipulation and genetic traceability. In addition, yeast is easy to grow and maintain and is stable in both the haploid and diploid state. As with other organisms, isotope labeling, whether by metabolic incorporation or by covalent tagging, offers a way to quantitatively compare proteomes between differentially treated samples in order to gain additional insight into the yeast proteome and biological processes present in eukaryotic cells. Some researchers use yeast to over-express labeled protein to obtain properly folded eukaryotic protein that may contain some post-translational modifications.

CIL is pleased to offer labeled cell growth media for *E.coli*, insect cells, yeast and eukaryotic cells. Specific human proteins may be over-expressed in a variety of cell types using these media in conjunction with recombinant techniques so that one can obtain a relatively large amount of labeled purified protein for proteomic studies. **Please see page 138 for a complete listing of yeast media and reagents.**

Insect Cell Media

The Baculovirus Expression Vector System (BEVS), first introduced in the mid-1980s, has grown to become the most versatile and widely used eukaryotic vector system employed for the expression of recombinant proteins in cultured insect cells. The BEVS is based on the infection of insect cells with recombinant baculovirus (BV) carrying the gene of interest with the subsequent expression of the corresponding recombinant protein by the insect cells. The most popular insect cell lines used in conjunction with the BEVS are Sf9 (*Spodoptera frugiperda*) and High Five™ (*Trichopulsia ni*).

CIL is proud to offer BioExpress® 2000, a rich growth media for culturing insect cells. The use of experimental design in the optimization of protein yield using BioExpress® 2000 is exemplified in CIL Application Note 20 at www.isotope.com.

BioExpress® 2000 is packaged as two components: a solid powder (a proprietary blend of inorganic salts, carbohydrates and labeled amino acids) and a liquid component (fatty acid solution). Selective amino acid-type labeling is possible with BioExpress® 2000 because the amino acid content is chemically defined. **Please see page 138 for a complete listing of BioExpress® 2000 products.**

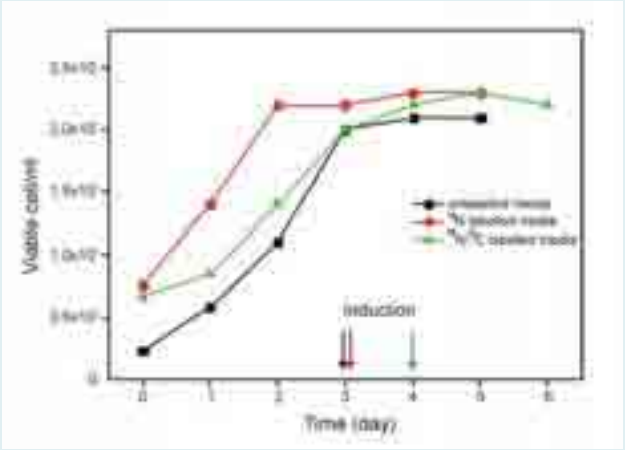
BioExpress® is a registered trademark of Cambridge Isotope Laboratories, Inc.

Mammalian Cell Media

There is continued interest in obtaining labeled recombinant protein from mammalian cells because eukaryotic protein expressed in mammalian cells has the greatest probability of being properly folded and functional.

CIL offers the only commercially available labeled mammalian media intended for the production of labeled protein with yields suitable even for NMR studies. Similar growth characteristics should be obtained using BioExpress® 6000 as with using Dulbecco's Modified Eagle Medium (DMEM). The amino acid content in BioExpress® 6000 is chemically defined so many different custom labeling strategies may be realized. Please see the growth curves below for HEK293 cells cultured in unlabeled, ^{15}N-labeled and $^{13}C,^{15}N$-labeled BioExpress® 6000. **Please see page 138 for a complete listing of BioExpress® 6000 products.**

BioExpress® is a registered trademark of Cambridge Isotope Laboratories, Inc.



Number of viable cells per mL of culture for differently labeled CIL media. Cells induced on day three and four and harvested two days later. No differences in cell densities are seen. Protein yield is approximately 2.2 mg/L cell culture in all cases. Data provided by Professor Harold Schwabe, Karla Warner and Professor Judith Klein-Seetharaman.

Refer to www.isotope.com for protocol on uniform isotope labeling of proteins with BV-infected Sf9 cells or scan the QR code below.

Cell-Free Protein Expression

CellFree Sciences (CFS) Products and Kits for Cell-Free Protein Expression

Producing proteins at will, often a bottleneck in post-genome studies, has become a reality with the advent of the robust wheat germ cell-free protein expression system. CellFree Sciences' ENDEXT® wheat germ cell-free system permits both high throughput protein screening and microgram- to milligram-scale protein production overnight. Protein synthesis protocols for the ENDEXT® system have been optimized for a wide range of proteins. They have also been incorporated into robotic protein synthesizers, versatile Protemist® DT II and mass-producing Protemist® XE. Being eukaryotic and free from physiological constraints that hamper *in vivo* systems, the wheat germ cell-free system allows synthesis, with or without additives, of a broad spectrum of protein and protein complexes ranging from 10 kDa to 360 kDa in well-folded and soluble forms.

The CFS wheat germ cell-free system was used to produce a large number of human proteins that are listed in the Human Gene and Protein Database (hgpd.lifesciencedb.jp). Expressed proteins were detected in almost all cases when CFS' wheat-germ extract and reagents were used.[1]

Please contact CFS directly (tech-sales@cfsciences.com) if you would like to use CFS's lab services to prepare a pEU plasmid with your target gene sequence, characterize the yield and solubility of your expressed protein, or produce a prescribed amount of protein using the wheat germ cell-free system.

1 Goshima, N.; Kawamura, Y.; Fukumoto, A.; Miura, A.; Honma, R.; Sato, R.; Wakamatsu, A.; Yamamoto, J.; Kimura, K.; Nishikawa, T., *et al.* **2008**. Human protein factory for converting the transcriptome into an *in vitro*-expressed proteome. *Nature Methods, 5,* 1011-1017.

Now Available!

Premium PLUS Expression Kit for MS

Premium PLUS Expression Kit for MS is a wheat germ cell-free protein expression kit for generating full-length, heavy labeled protein as MS internal standards. The expression of heavy labeled protein with more than 90% isotope incorporation efficiency for L-Lysine•2HCl ($^{13}C_6$ or $^{13}C_6$, $^{15}N_2$) and /or L-Arginine•HCl ($^{13}C_6$ or $^{13}C_6$, $^{15}N_4$) is easily achieved. This kit includes all the reagents necessary for transcription and translation as premixes for 16 reactions.

ENDEXT® is a registered trademark of CellFree Sciences.
Protemist® is a registered trademark of Emerald BioSystems.

Premium PLUS Expression Kit

Catalog No.	Description
EDX-PLUS-MS	Premium PLUS Expression Kit for MS

Please see pages 140-141 for a complete listing of CFS products and kits.

CIL is a distributor of the above-referenced CFS products in the US and Europe.

Cell-Free Protein Expression

CIL offers a wide variety of products for cell-free protein expression. Cell-free protein expression methods offer several advantages over expression using *E. coli* or other *in vivo* expression systems. These advantages include increased speed, ability to express toxic proteins, ease of amino acid type selective labeling and an open system that allows cofactors, chaperones, redox molecules and detergents to be easily be added to the expression system. Cell-free methods also allow co-expression of multiple proteins and are amenable to automation.

CIL is proud to distribute a wide range of products from CellFree Sciences (CFS). CIL also offers algal-derived amino acid mixes and conveniently packaged sizes (25 mg, 50 mg, 100 mg, *etc.*) of individual crystalline amino acids.

Amino Acid Mixes for Cell-Free Protein Expression

Catalog No.	Description
CLM-1548	Algal amino acid mixture (16AA) (U-^{13}C, 97-99%)
DLM-2082	Algal amino acid mixture (16AA) (U-D, 98%)
NLM-2161	Algal amino acid mixture (16AA) (U-^{15}N, 98%)
CNLM-452	Algal amino acid mixture (16AA) (U-^{13}C, 97-99%; U-^{15}N, 97-99%)
DNLM-819	Algal amino acid mixture (16AA) (U-D, 98%; U-^{15}N, 98%)
CDNLM-2496	Algal amino acid mixture (16AA) (U-^{13}C, 97-99%; U-D, 97-99%; U-^{15}N, 97-99%)
ULM-2314	Algal amino acid mixture (16AA) (unlabeled)
DLM-6819	"Cell Free" amino acid mix (20AA) (U-D, 98%)
NLM-6695	"Cell Free" amino acid mix (20AA) (U-^{15}N, 96-98%)
CNLM-6696	"Cell Free" amino acid mix (20AA) (U-^{13}C, 97-99%+; U-^{15}N, 97-99%)
DNLM-6818	"Cell Free" amino acid mix (20AA) (U-D, 98%; U-^{15}N, 98%)
CDNLM-6784	"Cell Free" amino acid mix (20AA) (U-^{13}C, 97-99%; U-^{15}N, 97-99%; U-D, 97-99%)
ULM-7891	"Cell Free" amino acid mix (20AA) (unlabeled)

Profiles for 16 Amino Acid Mixture (16 AA)		Profiles for 20 Amino Acid Mixture (20 AA)	
Approximate percentages, subject to lot-to-lot variability.			
L-Alanine	7%	L-Alanine	6%
L-Arginine	7%	L-Arginine	6%
L-Aspartic acid	10%	L-Asparagine	5%
L-Glutamic acid	10%	L-Aspartic acid	8%
Glycine	6%	L-Cysteine	3%
L-Histidine	2%	L-Glutamic acid	9%
L-Isoleucine	4%	L-Glutamine	5%
L-Leucine	10%	Glycine	5%
L-Lysine	14%	L-Histidine	1%
L-Methionine	1%	L-Isoleucine	3%
L-Phenylalanine	4%	L-Leucine	9%
L-Proline	7%	L-Lysine	12%
L-Serine	4%	L-Methionine	1%
L-Threonine	5%	L-Phenylalanine	4%
L-Tyrosine	4%	L-Proline	5%
L-Valine	5%	L-Serine	4%
		L-Threonine	4%
		L-Tryptophan	3%
		L-Tyrosine	3%
		L-Valine	4%

CIL does not provide protocols for formulation of amino acid mixtures, as the formulations may vary depending on application and reaction scale. For first-time amino acid formulations, the pH should be checked prior to use.

^{18}O Labeling

Dr. Catherine Fenselau
University of Maryland, College Park, MD 20742 USA

$^{18}O_2$-labeling is the Linux of isotope-labeling methods. Any laboratory can buy Water (^{18}O, 99%) (OLM-240-99) and adapt the method to its own applications. It offers a universal strategy for uniform labeling of all peptides from any kind of protein, including modified proteins.[1] It is used to label clinical samples with unrivaled sensitivity.[2, 3] The only byproduct is water, and the immobilized catalytic enzyme can be removed mechanically. Labeling with $^{18}O_2$ is limited to binary comparisons or series thereof, and it requires a workflow with minimal manipulation of proteins since the light and heavy samples are combined at the peptide level.

Two atoms of ^{18}O are introduced into the carboxylic acid group of every proteolytic peptide in a protein pool that has been catalyzed by members of the serine protease family, which includes trypsin, Glu-C protease, Lys-C protease and chymotrypsin. In the binding site of each protease, the residue of choice is covalently bound in a tetrahedral intermediate, which is then disrupted by nucleophilic attack by a water molecule, cleaving the protein. The C-terminal residue in each peptide product is re-bound by the protease, *e.g.* Arginine and Lysine in the case of trypsin, and released by hydrolysis. If the peptide products are incubated with the catalytic enzyme in Water (^{18}O, 99%) the level of ^{18}O in the peptides will eventually equilibrate with the level of ^{18}O in the solvent, preferably >95%.

Peptide binding by the protease offers the advantage that cleavage of the protein can be optimized and carried out separately from labeling the peptide.[4]

Each heavy peptide weighs 4 Da more than its $^{16}O_2$ light analog. After labeling, the mixtures of heavy and light peptides are mixed, and isotope ratios of peptide pairs are determined by LC/MS. Concurrent MS/MS measurements and appropriate computer algorithms can provide peptide identification along with quantitation.

References

1. Fenselau, C.; Yao, Z. **2009**. $^{18}O_2$-Labeling in quantitative proteomic strategies: a status report. *J Proteome Res, 8*, 2140-2143.
2. Zang, L.; Palmer Toy, D.; Hancock, W.S.; Sgroi, D.C.; Karger, B.L. **2004**. Proteomic analysis of ductal carcinoma of the breast using laser capture microdissection, LC-MS and $^{16}O/^{18}O$ isotopic labeling. *J Proteome Res, 3*, 604-612.
3. Bantscheff, M.; Dumpelfeld, B.; Kuster, B. **2004**. Femtomol sensitivity post-digest ^{18}O labeling for relative quantification of differential protein complex composition. *Rapid Commun Mass Spectrom, 18*, 869-876.
4. Yao, X.; Afonso, C.; Fenselau, C. **2003**. Dissection of proteolytic ^{18}O labeling: endoprotease-catalyzed ^{16}O-to-^{18}O exchange of truncated peptide substrates. *J Proteome Res, 2*, 147-152.

Enzymatic Labeling Products

The incorporation of two ^{18}O atoms into each C-terminus of peptides derived from proteolytic digestion of biological samples has emerged as one of the leading global labeling strategies used in comparative quantitative proteomics. The success of the technique is due in part to the relatively low cost of ^{18}O water, the resulting +4 Da mass increase in molecular weight for the "heavy" peptide and co-elution of $^{18}O/^{16}O$ peptide pairs from reverse-phase HPLC.

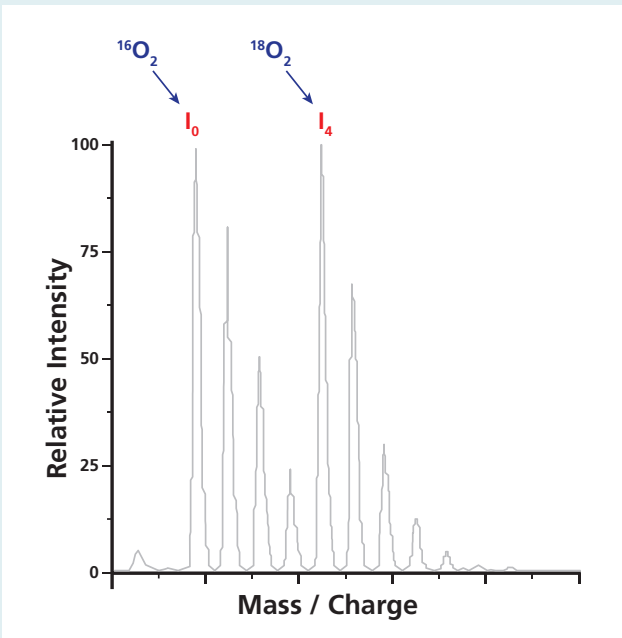
CIL is pleased to offer Water (^{18}O, 99%). This highly enriched material allows for the most complete labeling of peptides for proteomic applications.

Water (^{18}O)

Catalog No.	Description
OLM-240-97	Water (^{18}O, 97%)
OLM-240-99	Water (^{18}O, 99%)

Water (^{17}O)

Catalog No.	Description
OLM-782-90	Water (^{17}O, 90%)
OLM-782-70	Water (^{17}O, 70%)



Mass Spectrometry Signal Calibration for Protein Quantitation

Michael J. MacCoss, PhD
Associate Professor of Genome Sciences
University of Washington, School of Medicine, Seattle, WA 98195 USA

Introduction

Quantitative analysis of proteins and peptides by mass spectrometry is an important and growing area of biomedical research. Immunoassays have been the primary tool for protein quantitation. However, immunoassays are far from a perfect solution and may no longer meet the basic research needs in protein detection and quantitation. Recent efforts to overcome the limitations of immunoassays have shown that mass spectrometry assays, when combined with stable isotope-labeled internal standards, careful analyte calibration, quality control, and in some cases enrichment, can overcome the limitations of immunoassays. This is promising because it suggests that protein biochemists may no longer need to rely on the slow and expensive development of immunoassays for their target protein of interest. Instead, we can rely on mass spectrometry to deliver the sensitivity and specificity needed for the next generation of quantitative protein measurements at a greatly reduced cost. While the strengths of mass spectrometry are clear, these are still complicated measurements to perform.

Mass spectrometry has a long history of making quantitative measurements and has even been used for the quantitation of peptides for greater than three decades. However, in the proteomics field, we tend to use the term "quantitation" broadly. Frequently, methods described in papers that measure a signal intensity for peptides between two or more samples or conditions tend to be be labeled as quantitative. Are these data quantitative? Maybe, but not necessarily.

Here we will review some of the fundamentals of quantitative analysis and revisit what types of validation are required to assess whether a measurement is quantitative. We will review a few common strategies for the use of stable isotope-labeled internal standards in proteomics and how these data are used to calibrate the mass spectrometer response. We will approach this from a purely theoretical basis and enable the reader to assess whether the respective strengths and caveats should alter their chosen methodology depending on the intended application. Finally, we hope to correct a couple of misconceptions in the community about the use of stable isotope-labeled internal standards and what makes a mass spectrometry assay quantitative.

What Is the Difference Between Quantitative and Differential Analysis?

What makes any assay quantitative? To make a measurement quantitative, there must be a change in signal that reflects the change in quantity. To assess whether we get an expected change in signal with a change in quantity, we use standards of "known" quantity. We place quotations around known because the accuracy of any quantitative measurement is only going to be as good as the accuracy of the standards.

An illustration of a quantitative calibration curve can be seen in Figure 1. In this figure, the X-axis is the known quantity of standard samples and the Y-axis is the measured signal intensity. All measurements when plotted in this manner should have a range where there is a linear response between the measured signal intensity and the quantity of the target analyte. There will be a point at the lower end of this curve where the response in signal is not reflective of the quantity. This point is known as the lower limit of quantitation (LLOQ). There is also a similar point at the upper end of the linear dynamic range where the detector begins to saturate and no longer respond linearly (upper limit of quantitation or ULOQ). Quantitation should be performed in the linear dynamic range or at least in a range where there is a change in signal that reflects the change in quantity.

While it may be possible to obtain relative quantities without the use of a standard curve, it is impossible to assess whether all points are within the linear dynamic range of the instrument

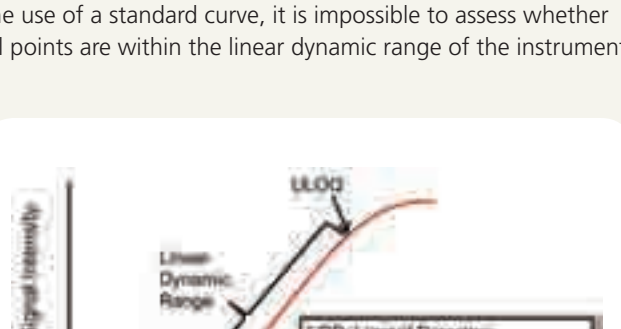


Figure 1. Illustration of a simplistic calibration curve.

without standards. Imagine two measurements, one below the LLOQ and one above. While there could be a statistically significant difference in intensity between the two measurements, the magnitude of the differences will not necessarily reflect a quantitative difference. Thus, without validation that the intensities are within the quantitative range, the measurements are limited to being differential and should not be considered quantitative.

Why Use a Stable Isotope-Labeled Internal Standard?

For the quantitation of compounds in complex matrices the use of internal standards minimizes errors associated with sample isolation and preparation because the compound of interest is measured relative to the added internal standard. A standard is chosen that will mimic the measured compound during the sample isolation and preparation, therefore will account for any possible losses.

The measurement of the ion-current ratio between the target peptide and an internal standard with a mass spectrometer significantly reduces errors associated with the ion source and inlet systems because "like molecules" will experience similar biases during the sample preparation and measurement. The use of stable isotopically labeled internal standards and isotope ratios further minimizes these errors and reduces the effect of long term drifts by using a standard, which is structurally identical to the peptide of interest. Therefore, when used with mass spectrometric detection, stable isotopically labeled proteins or peptides are a nearly ideal internal standard.

When performing quantitation with a stable isotope-labeled internal standard, the basic rules of quantitative analysis remain the same. The one difference is that instead of measuring a raw measured signal intensity, the intensity reported is a intensity normalized to the internal standard – essentially an ion current ratio. Thus, one divides the target signal by the intensity of the signal from the respective stable isotope-labeled internal standard (Figure 2). The use of the internal standard will not necessarily alter the quantitative accuracy or dynamic range but it should almost certainly improve the quantitative precision by minimizing sample preparation "noise".

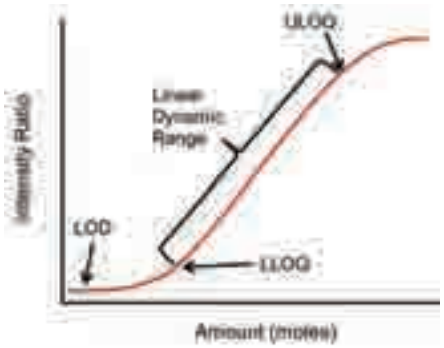


Figure 2. Illustration of a calibration curve using a stable isotope-labeled internal standard. The curve is identical to the one shown in Figure 1 except the Y-axis has been changed from a RAW signal intensity to a normalized intensity ratio.

In the example shown in Figure 2, the internal standard is used to normalize the signal intensity and improve the precision of the measurement. We want to make it clear that the stable isotope-labeled internal standard is not necessarily what makes the assay quantitative. The quantitation is still made relative to unlabeled standards. We do not need to know the amount of the stable isotope labeled internal standard particularly well – we just need to make sure that the same amount of the internal standard is added to every sample and standard.

Single-Point Calibration

Arguably the most common method used in proteomics for calibrating the instrument response is the use of a single-point calibration. In these experiments, a known quantity of a stable isotope-labeled peptide is added to a sample and then the signal of the target analyte is measured relative to the internal standard. The measured ratio *(R)* between the unlabeled peptide and internal standard is assumed to be proportional to the mole ratio between the two respective isotopomers

$$R \approx n_a / n_b \qquad \text{Equation 1}$$

where n_a and n_b are the moles of the unlabeled and stable isotope-labeled internal standard respectively. Assuming this relationship is linear, we can write a simple linear equation describing the relationship between the isotopomer mole ratio and the measured signal ratio in the mass spectrometer.

$$R = k \cdot n_a / n_b + R_b \qquad \text{Equation 2}$$

In this case, k is a response factor that can be used as a factor to correct differences in the response between the labeled and unlabeled peptide. Likewise, R_b is the measured background ratio during the injection of a blank that contains only the stable isotope-labeled internal standard and no endogenous peptide.

In these experiments it is assumed that $k = 1$ and $R_b = 0$ and standards are not necessarily run to confirm these assumptions over the range of the quantitative measurement. Given these assumptions and the "known" quantity of the stable isotope-labeled internal standard, then Equation 2 is rearranged to

$$n_a = R \cdot n_b \qquad \text{Equation 3}$$

to solve for the absolute quantity of endogenous peptide.

(continued)

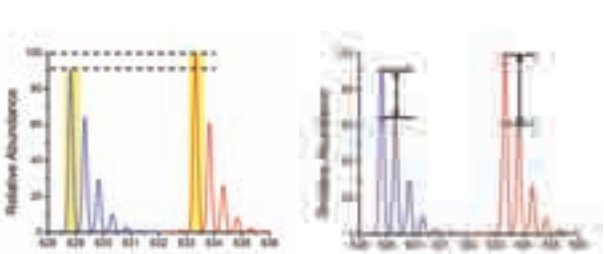


Figure 3. Theoretical effect of the measured mass spectrometer signal intensity between a peptide that contains only natural abundance isotopes and the identical peptide sequence that is enriched with 8 x ^{13}C atoms at 99.9 atom percent excess (APE)

This approach is used throughout the proteomics literature for "absolute" quantitation. However, is the assumption valid that k = 1 and R_b = 0? Should we expect equal quantities of unlabeled and stable isotope-enriched peptides to respond similarly in the mass spectrometer? The answer is, of course, it depends.

One main challenge that needs to be accounted for in the use of a simple single-point calibration using ^{13}C-labeled peptides is the effect on the isotope distribution (Figure 3). Consider the peptide sequence YAGILDC$_{ICAT}$FK where the cysteine residue is labeled with either an isotope-coded affinity tag (ICAT) reagent that contains natural abundance isotopes (light version) or nine of the carbons replaced with ^{13}C-enriched atoms at 99.9 APE (heavy version). If the two peptides are mixed at a perfect 1-to-1 mole ratio and the monoisotopic isotope peak is used for each of the two isotopomers the expected signal would not be 1-to-1 (Figure 3). In fact, assuming perfect and equal ionization between these two isotopomers, we would expect that the ^{13}C-labeled version of the peptide would actually be more intense by almost 10%. The signal intensity of the mono-isotopic mass of a stable isotope-labeled peptide should always be more intense than the unlabeled equivalent and the magnitude of the difference will depend on the elemental composition of the molecule, the number of labeled atoms, the type of labeled atom and the enrichment of the isotope-labeled starting material.

So what is the cause of this difference in signal intensity? This difference is caused because the monoisotopic peak is now a greater portion of the total isotope distribution in the heavy peptide relative to the light peptide. In the right side of Figure 3, it is obvious that the difference in intensity between the M+0 and the M+1 isotope is very different between the light and the heavy isotopomer. Interestingly, this effect is worse with ^{13}C labeling than ^{15}N, ^{18}O or D. This effect is because we are in essence removing nine carbons from the contribution to the M+1 isotope peak. At low resolution this is less of a problem because without resolution of the individual isotope peaks, the entire distribution is used and, in that case, the sum of the distributions are equal. Thus, in using high resolution mass spectrometry, Equation 3 cannot be used for absolute quantitation without applying a correction factor for the difference in the fraction of the monoisotopic peak of the total isotope distribution between the light and the heavy peptide. This correction factor can be computed theoretically for each light and heavy peptide or can be bypassed using a standard curve of known unlabeled peptide quantities (see below).

There are other limitations in the use of a single-point calibration with a stable isotope-labeled peptide standard. One is the lack of day-to-day reproducibility in the quantitative measurement (Hoofnagle *et al. Clin. Chem*). However, another major limitation is that with a single point, it is impossible to determine whether the signal intensities from both the light and the heavy peptide are both within the linear quantitative range of the mass spectrometer (Figure 1). As mentioned previously, the inability to confirm the measurements are within the linear range generally means the measurement should simply be considered differential and not quantitative. This being said, the simplicity of a single-point calibration still makes it an extremely popular and powerful strategy. However, it is important for users to be aware of the potential limitations in the method and/or data reported using this approach.

Using a Peptide Standard Curve

An improvement to the use of a single-point calibration is to use a peptide standard curve. In this case, a constant amount of a stable isotope-labeled internal standard peptide is added to several unlabeled peptide standards of known quantity that span the range of quantities that need to be quantified. The ratio of the target peptide signal is measured relative to the stable isotope-labeled internal standard *(R)* and plotted relative to the moles from the unlabeled peptide standards *(n_a)*. An example of a theoretical standard curve is shown in Figure 4. The linear portion of the curve follows the equation:

$$R = k \cdot n_a + R_b \qquad \text{Equation 4}$$

Where k is the slope of the standard curve and R_b is the background ratio from the injection of a blank containing only internal standard peptide. Unlike the single point calibration described above, the use of a peptide standard curve confirms that the measurement is within a linear range of quantitation.

A key advantage of the use of a peptide standard curve is that there is less demand on the chemical purity and isotope enrichment of the standards. As long as the same amount of internal standard is added to each standard and sample, the k in Equation 4 will correct for this appropriately. While it is helpful to know the absolute quantity of the stable isotope-labeled internal standard added to each sample, it is not

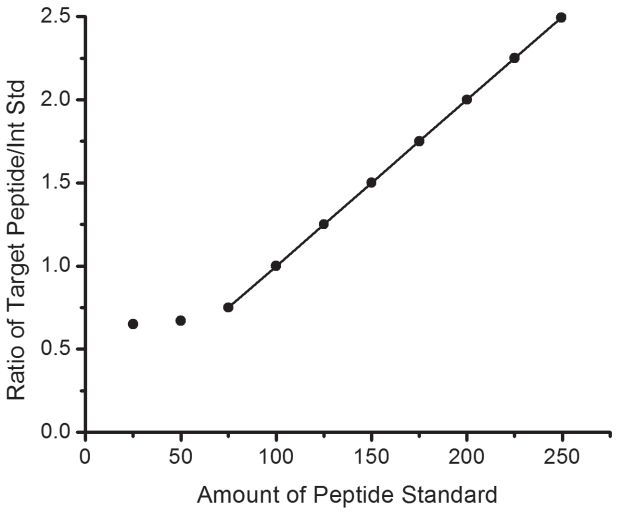


Figure 4. Theoretical calibration curve using known *peptide* standards. In this example, the amount of the peptide standard is plotted on the x-axis and the ratio of the unlabeled peptide relative to the respective stable isotope-labeled internal standard is used as the signal intensity. It is important that the amount of the stable isotope-labeled internal standard is the same across all samples but knowing the exact quantity of the internal standard is not essential. The two lowest abundance standards are not above the lower limit of quantitation.

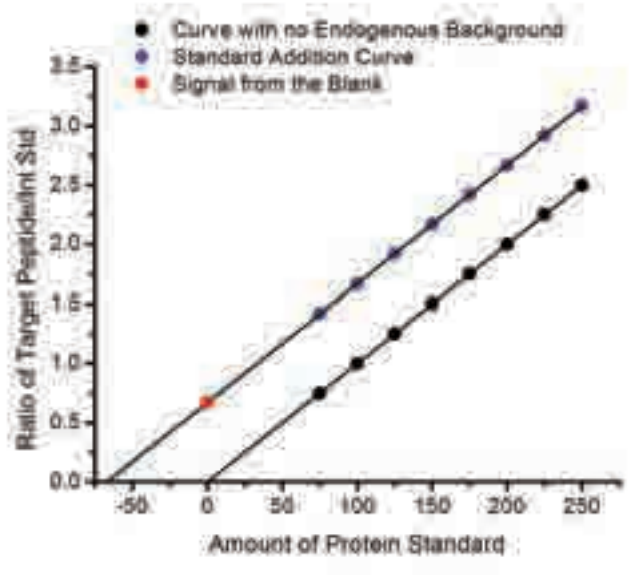


Figure 5. Theoretical calibration curves using known *protein* standards. In these examples, the amount of the standard is plotted on the x-axis and the ratio of the unlabeled peptide relative to the respective stable isotope-labeled internal standard is used as the signal intensity. In the black example, the protein standards are in a sample matrix without any endogenous protein. The blue example is a standard curve that adds the standards to the sample matrix where there is an endogenous amount of the target protein. The red point is the signal measured from the endogenous matrix when no additional protein standard is spiked into the sample. The slope of the two lines should be indistinguishable.

necessary. Thus, it is more important to have peptide standards at high purity and absolute known quantities for the unlabeled peptide than it is for the stable isotope-labeled peptides. While the use of a peptide standard curve offers advantages over a single point calibration, it still has limitations. The main thing that the user needs to consider is that the instrument response is calibrated relative to *peptide* standards and, therefore, it is important to be careful about making claims about *protein* quantities. While the quantitation of peptides can be made very accurate and precise, these measurements do not account for incomplete recovery of peptides during the sample preparation and digestion of the protein sample.

Using a Protein Standard Curve

If the goal is to quantify proteins then it is best to use actual protein standards with known quantity to calibrate the signal response. We recently described an inexpensive way to generate protein standards and determine their quantity using *in vitro* transcription/translation (Stergachis *et al. Nat. Methods* 2011). By using an actual protein standard, the sample is then calibrated relative to the protein and not the peptide (Figure 5). The use of a protein standard is better than the use of a peptide standard because it has to undergo digestion to produce the peptide that is measured just like the endogenous protein.

While a recombinant protein is a better standard than a peptide, the measured peptides from the protein standard still might not represent what is measured from the endogenous protein. One complication is that a protein standard will likely be prepared from a sample matrix that is different from the endogenous sample matrix. In this case, a protein might experience different digestion efficiency in the sample relative to a standard buffer. A way to minimize this sample preparation difference is to spike the standards of varying concentration into the same background sample (see Figure 5 blue curve). This approach is known as the method of standard addition and the standard curve should now reflect differences in sample preparation and measurement that are reflective of the sample matrix. The curve from the standard addition in the sample matrix will often have the same slope as the curve with no endogenous background, but the intercept will be higher because of endogenous amount of the protein. If the endogenous quantity is within the linear quantitative range of the measurement, the line from the spiked standards will go through the signal measured from the sample with only the

(continued)

endogenous quantity of protein (Figure 5, red point). The absolute amount of the endogenous peptide in the background matrix can be estimated using the negative X-intercept.

Conclusions

There are many different ways to calibrate the instrument response in quantitative mass spectrometry. Here we described three common ways of performing signal calibration for proteomics. Each of the methods has different positive and negative attributes. The user should be aware of the limitations of the method that they choose and, thus, interpret their data appropriately. Depending on the scale of the experiment, the required accuracy and precision, and the reagent budget, the user can choose the best approach for the project.

References

Hoofnagle *et al.* **2010**. *Clin Chem, 56,* 1561-70.

Stergachis, A.B.; MacLean, B.; Lee, K.; Stamatoyannopoulo, J.A.; MacCoss, M.J. **2011**. Rapid empirical discovery of optimal peptides for targeted proteomics. *Nature Methods, 8,* 1041–1043. 10.1038/nmeth.1770.

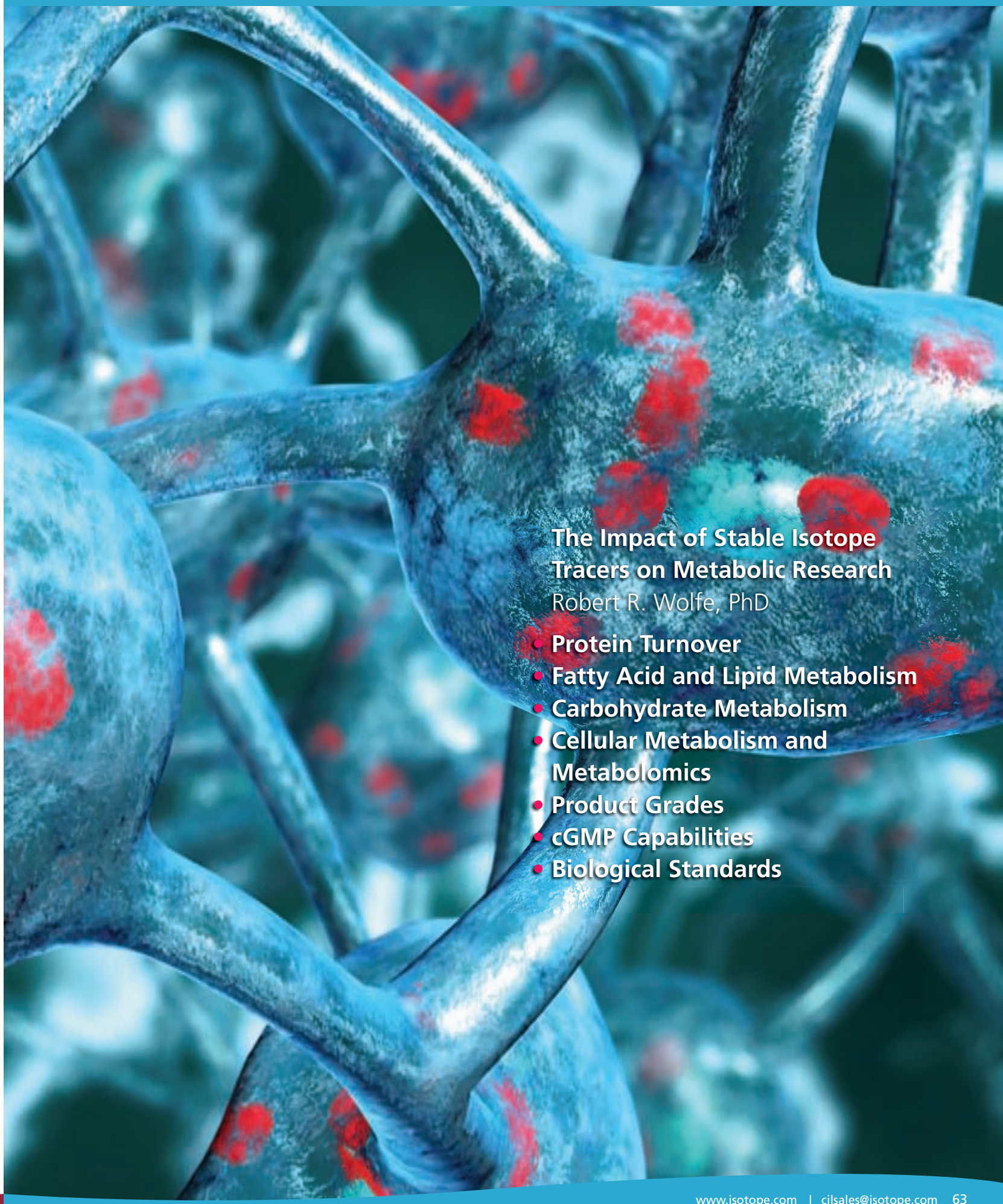
Useful Software Tools for the Analysis and Management of Mass Spectrometry Data

Skyline – http://skyline.maccosslab.org
Skyline is a freely available and open-source Windows client application for Selected Reaction Monitoring (SRM)/ Multiple Reaction Monitoring (MRM) and Full-Scan (MS1 and MS/MS) quantitative methods and analyzing the resulting mass spectrometer data. It makes use of cutting-edge technologies for creating and iteratively refining targeted methods for large-scale proteomics studies.

Panorama – http://panoramaweb.org
Panorama is a freely available, open-source repository server application for targeted proteomics assays that integrates into a Skyline proteomics workflow. PanoramaWeb is a public Panorama server hosted at the University of Washington where laboratories and organizations can own free projects. You can request a project on this server to find out what Panorama has to offer, without having to set up and maintain your own server. You will be able to explore all the available features in Panorama, and be given administrative rights to your project so that you can set up folders and configure permissions. Panorama can also be installed by laboratories and organizations on their own servers.

Topograph – http://topograph.maccosslab.org
Topograph is a Windows application designed to analyze data in protein turnover experiments. Protein turnover experiments involve modifying an organism's diet or growth media to include a stable isotope-labeled amino acid, and then measuring the rate at which the label appears in peptides.

CHORUS – http://chorusproject.org
CHORUS is an effort to provide a free, professionally developed community solution for the storage, sharing and analysis of mass spectrometry data. This is currently a collaborative partnership between the University of Pittsburgh, University of Washington, Infoclinika and Amazon Web Services. The application provides a "Google Docs" type interface optimized for mass spectrometry data. Data can be uploaded and kept private, shared with a group of collaborators, or made entirely public. Tools are available to visualize and analyze the data directly on the cloud.



The Impact of Stable Isotope Tracers on Metabolic Research

Robert R. Wolfe, PhD

- Protein Turnover
- Fatty Acid and Lipid Metabolism
- Carbohydrate Metabolism
- Cellular Metabolism and Metabolomics
- Product Grades
- cGMP Capabilities
- Biological Standards

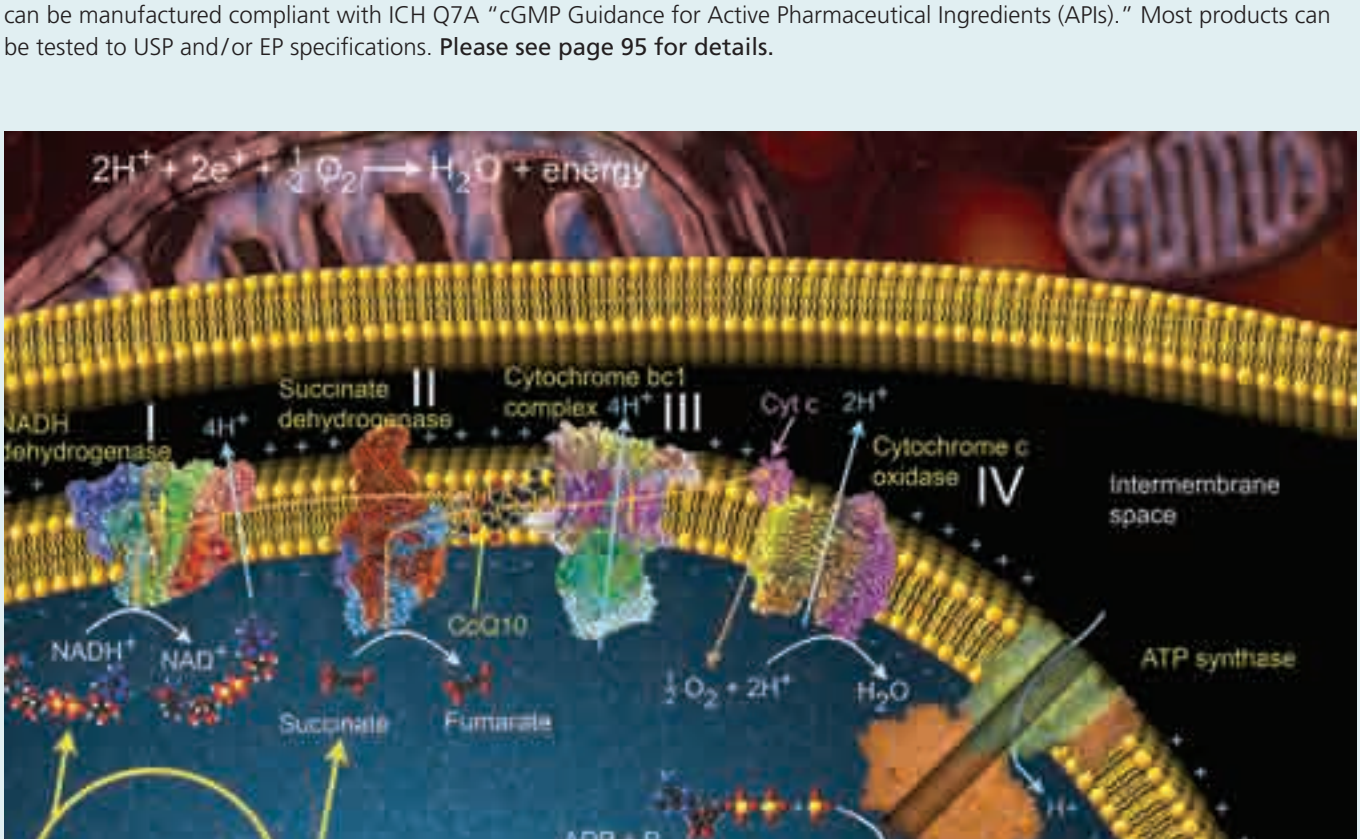
Metabolic Research

CIL offers the most complete listing of stable isotopically labeled metabolic substrates available. These substrates are labeled with carbon-13, deuterium, nitrogen-15, oxygen-18, as well as other stable isotopes. Some of the many applications for these compounds include the utilization of amino acids for protein turnover studies, carbohydrates for glucose metabolism studies and fatty acids for lipolysis research. Researchers utilize a number of different methods to study metabolism including mass spectrometry, MRI and MRS.

Isotope-dilution mass spectrometry (IDMS) is inarguably the most accurate, sensitive, reproducible and popular method available for quantifying small and intermediate-sized molecules in a wide range of sample types. One primary reason why compounds enriched in stable isotopes make ideal internal standards for comparative or absolute quantitation using mass spectrometry is that separate signals from the "heavy" (isotope-enriched) and "light" (native) forms of the same compound are detected simultaneously.

CIL recognizes the importance of high chemical purity and isotopic enrichment for metabolic studies and tests all products to meet high specifications for both. All products are shipped with a Certificate of Analysis that indicates the passing results and an MSDS to describe the physical characteristics and safety of the product. CIL may be able to provide additional data upon request. CIL can also offer products that are microbiological tested. An Enhanced Technical Data Package (EDP) is available for some microbiological-tested products (MPT). **Please see pages 92-93 for a complete listing of MPT products and page 94 for a complete description of the EDP.**

CIL is able to manufacture compounds to current Good Manufacturing Practice (cGMP). A majority of the compounds in this listing can be manufactured compliant with ICH Q7A "cGMP Guidance for Active Pharmaceutical Ingredients (APIs)." Most products can be tested to USP and/or EP specifications. **Please see page 95 for details.**



The Impact of Stable Isotope Tracers on Metabolic Research

Robert R. Wolfe, PhD
Professor, Department of Geriatrics
Jane and Edward Warmack Chair in Nutritional Longevity
Reynolds Institute on Aging, University of Arkansas for Medical Sciences, Little Rock, AR 72223 USA

Tracer methodology has advanced the field of metabolism by enabling the quantification of metabolic reactions *in vivo*. Stable isotope tracers have been particularly important in this regard, as these tracers have made possible a wide range of studies that would not have been possible with radioactive tracers. Early efforts using stable isotope tracers focused on determining the nature of protein "turnover," or the simultaneous processes of protein synthesis and breakdown. As analytical techniques have developed and a wide variety of isotopic tracers have become available, the scope of tracer studies has widened to the point where it would be impossible to even touch on all possible applications. Some specific examples will be discussed.

Methods using stable isotope tracers fall into two general categories: those in which the use of stable isotopes is a preferable option to the use of the corresponding radioactive tracer for reasons of ease of disposal or analysis; and methods for which there are no radioactive tracers available that would enable quantification of the metabolic pathway under investigation.

The ease of disposal of stable isotopes stems from the fact that, unlike radionuclides, they do not undergo spontaneous decay with resulting emissions that have adverse biological effects (hence the name *stable* isotopes). Stable isotopes are naturally occurring and may be present in significant amounts. For example, slightly more than one percent of all naturally occurring carbon is ^{13}C, and the amount of ^{13}C infused in the context of a tracer study will likely not significantly affect the whole body level of enrichment. Since mice have been raised to have almost entirely ^{13}C in their bodies without apparent adverse effects, we can be quite confident that the experimental use of stable isotopes is safe and that no special procedures are necessary in the disposal of animals given stable isotopes. The potential analytical advantages of stable isotope tracers are two-fold. If mass spectrometry is used to measure enrichment, then the ratio of tracer to tracee is measured directly as opposed to the separate measurement of concentration and decays per minute (dpms) and the calculation of specific activity (the expression of tracer/tracee ratio when radioactive tracers are used). Another advantage of stable isotopes stemming from analysis is that the use of selected ion monitoring with mass spectrometry enables definitive proof that the analyte has been isolated in absolutely pure form for the measurement of stable-isotope enrichment.

Also, the measurement of enrichment in specific positions of a molecule is generally much more feasible with mass spectrometry and stable isotopes than the chemical isolation of radioactive atoms in specific positions in a molecule and subsequent determination of dpms of those isolated atoms. The use of D-Glucose (6,6-D_2, 99%) (DLM-349) to measure the rate of hepatic glucose production is the most common example of a stable isotope providing an alternative to the radioactive analogue.

While factors such as safety and convenience are important, the most exciting advances in metabolism stemming from the use of stable isotope tracers generally involve the quantification of metabolic pathways that realistically could not have been measured otherwise. Nitrogen metabolism is the most obvious example, since there is no radioactive isotope of nitrogen. Since nitrogen is the key element that defines amino acids and protein, a wide variety of applications have been derived to quantify various aspects of nitrogen metabolism in the body using ^{15}N as a tracer. Stable isotopes of carbon and hydrogen have also been used to label amino acids in order to perform novel studies of amino acids. Individual amino acids can be labeled with a variable number of heavy stable isotopes in order to produce molecules of different molecular weight that retain the same metabolic functions (isotopomers). Isotopomers can be useful in a variety of approaches. For example, measurement of muscle protein synthesis involves infusion or injection of an amino acid tracer, and measurement of the rate of incorporation over time into the tissue protein. Collection of the muscle protein requires a muscle biopsy. By staggering the times of administration of isotopomers of the same amino acid, one single biopsy can suffice to determine the rate of incorporation over time, thereby enabling the calculation of the rate of muscle protein synthesis.

Stable-isotope methodology also enables the concurrent use of the same isotope incorporated in to numerous molecules. Since there are 20 different amino acids in the body, it is often important to study the interaction of the kinetics of multiple amino acids. For example, amino acid transmembrane transport differs for specific amino acids, but there is overlap in carrier functions for particular amino acids. It is, therefore, advantageous to quantify transport rates of different amino acids simultaneously. This can be accomplished using stable isotope tracers of the amino acids of interest because the amino acids are separated by gas or liquid chromatography

(continued)

prior to measurement by mass spectrometry. Therefore, different amino acids with the same stable isotope tracer can be distinguished even though the mass increase caused by the tracer is the same in each case.

The unique ability to measure by mass spectrometry the enrichment of a variety of molecules enriched with the same stable isotope tracer has been central to the development of many new approaches in the field of metabolic research. The most popular method involves administering Deuterium oxide (D, 99.9%) (DLM-4) and measuring the synthetic rates of a wide variety of molecules by determining the rate of incorporation of the D. It is also possible to quantify intracellular reaction rates using both positional and mass isotopomers of the same tracer, most commonly using ^{13}C. Use of positional isotopomers to calculate various intracellular flux rates involves administering a molecule with labeling in a specific position and determining by mass spectrometry the extent of appearance of the stable isotope tracer in other positions of the same molecule, or in specific positions of other molecules. Mass isotopomers have proven useful to determine the enrichment of precursors of the synthesis of polymers such as fatty acids. If multiple labeled precursors (*e.g.* ^{13}C-acetate) are incorporated into a product that is a polymer of the precursor (*e.g.* palmitate), then this will be reflected in the mass increase in the product. From the profile of mass increases in the product the precursor for synthesis can be calculated.

Increased sophistication of mass spectrometry analysis has led to the development of the field of metabolomics. The concentrations of a wide variety of compounds, usually in the blood or urine, are measured to develop a profile distinctive of a particular metabolic state. Stable isotope tracers have played an important role in metabolomics, as their use as internal standards enable quantification of the concentration of any tracee for which a stable isotope tracer is available. Although the metabolomics approach has proven useful in some circumstances, there has been some ambiguity in interpreting metabolomics profiles because they reflect only concentrations. For that reason the field of fluxomics is evolving in which a wide variety of tracers are given to the subject before the blood is sampled so that the metabolomics profile can reflect not only concentrations, but also the flux rates of relevant metabolic pathways.

This brief overview is meant only as a introduction to the varied possibilities possible with the use of stable isotope tracers. A key factor in the development and advancement of these applications has been the increasing availability of a wide variety of stable isotope tracers from CIL. The diversity of the CIL products has advanced to the point where the application of new methodologies is limited only by our own insights and creativity.

Further information about stable isotope tracer methodology as applied to metabolic research can be learned at the annual course "Isotope Tracers in Metabolic Research" held in Little Rock, AR, organized by Drs. Bob Wolfe and Henri Brunengraber, and sponsored by the NIH. The course provides an intensive exposure to a variety of techniques and seasoned investigators in the field. For information about the course, please contact Deb Viane at djviane@uams.edu.

Related Products

Catalog No.	Description
DLM-349	D-Glucose (6,6-D_2, 99%)
DLM-4-70	Deuterium oxide (D, 70%)
DLM-4	Deuterium oxide (D, 99.9%)

Protein Turnover

The highest levels of accuracy and specificity for protein turnover studies are achieved using mass spectrometric detection and stable isotope-labeled amino acids as metabolic tracers. The use of labeled amino acids for metabolic incorporation allows for both quantitative and qualitative data on protein synthetic and degradation rates to be obtained. Specifically, labeled amino acids and other substrates are utilized to study whole body protein turnover, muscle protein synthetic rates, and the production and clearance of proteins and other biomolecules in the brain and cerebrospinal fluid. Below is a partial list of stable isotope-labeled substrates that can be utilized to study protein turnover. **For a full list of substrates, please see the product listing starting on page 129.**

Related Products

Catalog No.	Description
CLM-2265-H	L-Arginine•HCl ($^{13}C_6$, 99%)
NLM-395	L-Arginine•HCl (guanido-$^{15}N_2$, 98%+)
CNLM-539-H	L-Arginine•HCl ($^{13}C_6$, 99%; $^{15}N_4$, 99%)
NLM-6850	L-Citrulline (ureido-$^{15}N_1$, 98%)
CDLM-7139	L-Citrulline (5-^{13}C, 99%; 4,4,5,5-D_4, 95%)
DLM-4-70	Deuterium oxide (D, 70%)
DLM-4-99	Deuterium oxide (D, 99%)
DLM-4-99.8	Deuterium oxide (D, 99.8%)
DLM-4	Deuterium oxide (D, 99.9%)
DLM-556	L-Glutamic acid (2,3,3,4,4-D_5, 97-98%)
CLM-1166	L-Glutamine (5-^{13}C, 99%)
CLM-1822-H	L-Glutamine ($^{13}C_5$, 99%)
NLM-202	Glycine (^{15}N, 98%)
CLM-468	L-Leucine (1-^{13}C, 99%)
CLM-2262-H	L-Leucine ($^{13}C_6$, 99%)
DLM-1259	L-Leucine (5,5,5-D_3, 99%)
DLM-4212	L-Leucine (isopropyl-D_7, 98%)
DLM-567	L-Leucine (D_{10}, 98%)
CLM-653	L-Lysine•2HCl (1-^{13}C, 99%)
CLM-2247-H	L-Lysine•2HCl ($^{13}C_6$, 99%)
NLM-143	L-Lysine•2HCl (α-^{15}N, 95-99%)
DLM-2640	L-Lysine•2HCl (4,4,5,5-D_4, 96-98%)
CNLM-291-H	L-Lysine•2HCl ($^{13}C_6$, 99%; $^{15}N_2$, 99%)

Catalog No.	Description
CLM-206	L-Methionine (methyl-^{13}C, 99%)
CLM-893-H	L-Methionine ($^{13}C_5$, 99%)
CLM-762	L-Phenylalanine (1-^{13}C, 99%)
CLM-1055	L-Phenylalanine (ring-$^{13}C_6$, 99%)
DLM-1258	L-Phenylalanine (ring-D_5, 98%)
NLM-108	L-Phenylalanine (^{15}N, 98%)
CLM-2260-H	L-Proline ($^{13}C_5$, 99%)
CLM-441	Sodium bicarbonate (^{13}C, 99%) CP 97%+
DLM-449	L-Tyrosine (ring-3,5-D_2, 98%)
DLM-451	L-Tyrosine (ring-D_4, 98%)
CLM-311	Urea (^{13}C, 99%)
NLM-233	Urea ($^{15}N_2$, 98%)
DLM-488	L-Valine (D_8, 98%)
OLM-240-10	Water (^{18}O, 10%)
OLM-240	Water (^{18}O, 97%)

For a complete listing of labeled amino acids, please see pages 130-134.

Many of these products are available as microbiological and pyrogen tested. Please see pages 92-93 for a complete listing of these products.

"Life is sustained by constant dynamic metabolic adaptations to environmental changes in healthy and disease states. This is the reason why stable isotope-labeled compounds help us to understand kinetics modifications. Thus, we are looking for 'high quality' products which can be used safely. Thanks to CIL products, which are delivered quickly after order, with purity analysis and quality control, we have been able to propose new concepts in nutrition. Thus, we are satisfied by their products."

Professor Yves Boirie
Human Nutrition Unit – INRA / Université d'Auvergne
Human Nutrition Research Center of Auvergne
58 rue Montalembert, 63009 Clermont-Ferrand, France

Investigator Spotlight

Protein Turnover

Amy Claydon, PhD
Protein Function Group, Institute of Integrative Biology
University of Liverpool, Liverpool, UK L69 3BX

For an organism to respond to changes in its environment, the abundance of specific proteins must be altered. How rapidly this change can be brought about is controlled in part by the rate of turnover of the proteins; a protein that has a high rate of turnover can be increased in abundance, or removed from the protein pool, very rapidly. To determine the rate of protein synthesis using mass spectrometry, the incorporation of a stable isotope-labeled tracer into newly synthesized proteins can be monitored. Similarly, to assess rates of degradation, loss of the tracer is followed. One of the classes of tracer used to monitor flux through the protein pool is stable isotope-labeled amino acids.[1,2]

The Protein Function Group has always had a keen interest in proteome dynamics, initially in simple cellular systems, such as yeast and mammalian cell culture but also in more complex whole animal models. In one of the first studies of proteome dynamics, turnover rates of yeast proteins from cells grown in glucose-limiting conditions in a chemostat were determined using L-Leucine (D_{10}, 98%) (DLM-567) in the growth media[3,4] in an "unlabeling" experiment.[2] In a similar "dynamic-SILAC" study, human A549 adenocarcinoma cells were labeled with L-Arginine•HCl ($^{13}C_6$, 99%) (CLM-2265-H) and the rate of loss of label monitored for almost 600 intracellular proteins using a geLC/MS approach.[5]

In animal systems, the challenge becomes one of effective administration of the stable isotope tracer. We have taken a simple approach and administer label via incorporation into the diet. In a study of domestic fowl, a semi-synthetic diet was formulated for chickens, containing L-Valine (D_8, 98%) (DLM-488) at a relative isotopic abundance (RIA) of 0.5 (in other words, half of the valine was isotopically labeled). This might be seen as a compromise, but totally synthetic diets can be unpalatable and so partial labeling allowed design of a diet to sustain the high growth rate of the young chicks.[6] Proteins from skeletal muscle were assessed over the five-day labeling period and turnover rates were determined, after first calculating the RIA of the muscle precursor pool using multiply labeled peptides based on mass isotopomer distribution analysis.[7] For mice, we have used a semi-synthetic diet containing L-Valine (D_8, 98%) in which we simply added the stable isotope-labeled amino acid to the same level as that present in a standard laboratory chow. The rate of turnover of proteins in different organs (liver, kidneys, heart and skeletal muscle) was assessed – using major urinary proteins (MUPs) synthesised in the liver and excreted in the urine – to track the labeling trajectory non-invasively without the requirement for large numbers of animals.[8] The same diet has also been used in a different study, to track the baseline replacement of reproductive proteins from the epididymis and seminal vesicles of mice, to predict which proteins are mostly likely to respond under conditions of sperm competition.[9] As a further part of this study, a semi-synthetic diet containing L-Lysine•2HCl ($^{13}C_6$, 99%) (CLM-2247-H) at an RIA of 0.5 will be manufactured to assess the relative investment of different males mating under different levels of sperm competition.

The use of stable isotope-labeled amino acids enables the turnover trajectories of individual proteins in a diverse range of samples to be determined with relative ease, especially with the increasing software options for downstream data-analysis. We have shown that simple supplementation of a standard laboratory diet with an amino acid, at an RIA less than 1, is a cost-effective and biologically defendable approach to whole animal studies.

References

1. Beynon, R.J. **2005**. The dynamics of the proteome: strategies for measuring protein turnover on a proteome-wide scale. *Brief Funct Genomic Proteomic, 3*, 382-90.
2. Claydon, A.J.; and Beynon, R.J. **2012**. Proteome dynamics: revisiting turnover with a global perspective. *Molecular & Cellular Proteomics, 11,* 1551-1565.
3. Pratt, J.M., *et al.* **2002**. Dynamics of protein turnover, a missing dimension in proteomics. *Mol Cell Proteomics, 1*, 579-91.
4. Claydon, A.J.; and Beynon, R.J. **2011**. Protein turnover methods in single-celled organisms: dynamic SILAC. *Methods Mol Biol, 759*, 179-95.
5. Doherty, M.K., *et al.* **2009**. Turnover of the human proteome: determination of protein intracellular stability by dynamic SILAC. *J Proteome Res, 8*, 104-12.
6. Doherty, M.K., *et al.* **2005**. Proteome dynamics in complex organisms: Using stable isotopes to monitor individual protein turnover rates. *Proteomics, 5*, 522-533.
7. Doherty, M.K.; and and Beynon, R.J. **2006**. Protein turnover on the scale of the proteome. *Expert Rev Proteomics, 3*, 97-110.
8. Claydon, A.J., *et al.* **2012**. Protein turnover: measurement of proteome dynamics by whole animal metabolic labeling with stable isotope-labeled amino acids. *Proteomics, 12*, 1194-206.
9. Claydon, A.J., *et al.* **2012**. Heterogenous turnover of sperm and seminal vesicle proteins in the mouse revealed by dynamic metabolic labeling. *Mol Cell Proteomics, 11*, M111 014993.

Related Products

Catalog No.	Description
CLM-2265-H	L-Arginine•HCl ($^{13}C_6$, 99%)
DLM-567	L-Leucine (D_{10}, 98%)
CLM-2247-H	L-Lysine•2HCl ($^{13}C_6$, 99%)
DLM-488	L-Valine (D_8, 98%)

Determining Protein Turnover in Fish with D_7-Leucine

Mary K. Doherty,[1] Iain S. Young,[2] Simon J. Davies,[3] Phillip D. Whitfield[1]
1. Department of Diabetes and Cardiovascular Science, University of the Highlands and Islands, Inverness, UK
2. Institute of Integrative Biology, University of Liverpool, Liverpool, UK
3. Fish Nutrition and Health Research Group, School of Biological and Biomedical Sciences, University of Plymouth, Plymouth, UK

The proteome of a biological system is a dynamic entity and in constant flux (Doherty and Beynon, 2006). Different proteins turn over at distinctly different rates and even in a position of apparent steady-state, the protein complement is constantly changing. Moving from a static "snapshot" of a proteome to a dynamic view presents a considerable technical challenge, however, the utilization of stable isotope labeling of organisms in conjunction with mass spectrometry has led to considerable advances. These novel proteomic technologies have introduced the possibility of determining the turnover rates of multiple proteins in intact animal species including chicken and mice (Doherty *et al.*, 2005; Price *et al.*, 2010; Claydon *et al.*, 2011). We have extended this experimental strategy to measure the rates of synthesis and degradation of individual proteins in the skeletal muscle of fish (Doherty *et al.*, 2012) (Figure 1). In particular, we were interested in whether it was possible to distinguish the rates of synthesis of a family of isomeric proteins, β-parvalbumins. In our study, common carp were fed with an experimental diet in which 50% of the L-leucine in the diet was replaced with crystalline L-Leucine (isopropyl-D_7, 98%) (DLM-4212). Leucine was used as this is an essential amino acid and abundant in the carp proteome (Murai and Ogata, 1990). Importantly, the signature tryptic peptides from the individual parvalbumin β-isoforms all contain a leucine residue. The time-dependant incorporation of the isotope into parvalbumin isoforms was monitored by LC/MS analysis of the signature peptides and the data deconvoluted using mass isotopomer distribution analysis (Hellerstein *et al.*, 1992). Our data showed that the absolute rate of synthesis of parvalbumin β-isoforms in the skeletal muscle of common carp differed by an order of magnitude under steady-state conditions. Whilst the focus of our work was on specific isoforms, this approach can be used to determine the turnover of multiple proteins in carp tissues. The methodology may also be adapted to study proteome dynamics in different species of fish.

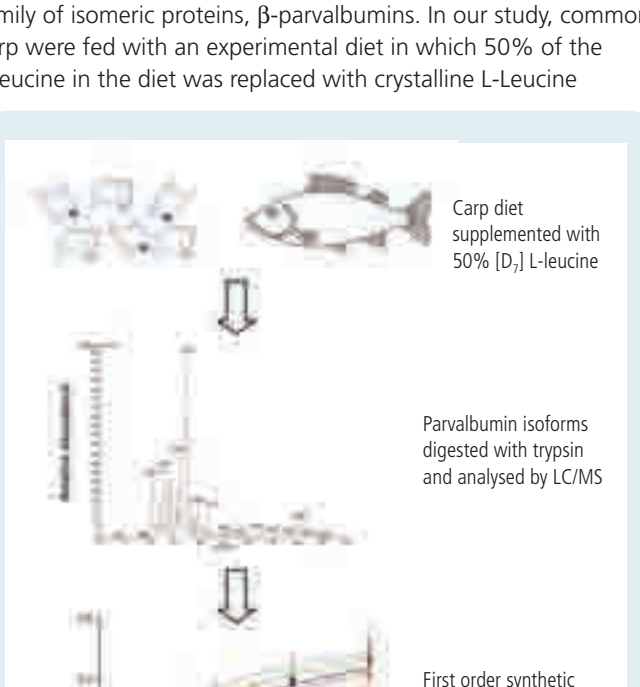


Figure 1.

Related Product

Catalog No.	Description
DLM-4212	L-Leucine (isopropyl-D_7, 98%)

References

Claydon, A.J.; Thom, M.D.; Hurst, J.L.; Beynon, R.J. **2012**. Protein turnover: measurement of proteome dynamics by whole animal metabolic labeling with stable isotope-labeled amino acids. *Proteomics, 12*: 1194-1206.

Doherty, M.K.; Beynon, R.J. **2006**. Protein turnover on the scale of the proteome. *Expert Rev Proteomics, 3*, 97-110.

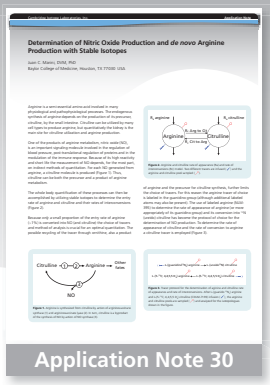
Doherty, M.K.; Brownridge, P.; Owen, M.A.; Davies, S.J.; Young, I.S.; Whitfield, P.D. **2012**. A proteomics strategy for determining the synthesis and degradation rates of individual proteins in fish. *J Proteomics, 75*, 4471-4477.

Doherty, M.K.; Whitehead, C.; McCormack, H.; Gaskell, S.J.; Beynon, R.J. **2005**. Proteome dynamics in complex organisms: using stable isotopes to monitor individual protein turnover rates. *Proteomics, 5*, 522-533.

Hellerstein, M.K.; Neese, R.A. **1992**. Mass isotopomer distribution analysis: a technique for measuring biosynthesis and turnover of polymers. *Am J Physiol, 263*, E988-1001.

Murai, T; Ogata, H. **1990**. Changes in free amino acid levels in various tissues of common carp in response to insulin injection followed by force-feeding an amino acid diet. *J Nutr, 120*, 711-718.

Price, J.C.; Guan, S.; Burlingame, A.; Prusiner, S.B.; Ghaemmaghami, S. **2010**. Analysis of proteome dynamics in the mouse brain. *Proc Natl Acad Sci USA, 107*, 14508-14513.



Determination of Nitric Oxide Production and *de novo* Arginine Production with Stable Isotopes

Juan C. Marini, DVM, PhD
Baylor College of Medicine, Houston, TX 77030 USA

Arginine is a semi-essential amino acid involved in many physiological and pathophysiological processes. The endogenous synthesis of arginine depends on the production of its precursor, citrulline, by the small intestine. Citrulline can be utilized by many cell types to produce arginine, but quantitatively the kidney is the main site for citrulline utilization and arginine production.

One of the products of arginine metabolism, nitric oxide (NO), is an important signaling molecule involved in the regulation of blood pressure, post-translational regulation of proteins and in the modulation of the immune response. Because of its high reactivity and short life the measurement of NO depends, for the most part, on indirect methods of quantitation. For each NO generated from arginine, a citrulline molecule is produced (see figure below). Thus, citrulline can be both the precursor and a product of arginine metabolism.

The whole body quantification of these processes can then be accomplished by utilizing stable isotopes to determine the entry rate of arginine and citrulline and their rates of interconversions (Figure 2).

Because *only* a small proportion of the entry rate of arginine (~1%) is converted into NO (and citrulline) the choice of tracers and method of analysis is crucial for an optimal quantitation. The possible recycling of the tracer through ornithine, also a product of arginine and the precursor for citrulline synthesis, further limits the choice of tracers. For this reason the arginine tracer of choice is labeled in the guanidino group (although additional labeled atoms may also be present). The use of L-Arginine•HCl (guanido-$^{15}N_2$, 98%+) (NLM-395) to determine the rate of appearance of arginine (or more appropriately of its guanidino group) and its conversion into L-Citrulline (ureido-$^{15}N_1$, 98%) (NLM-6850) has become the protocol of choice for the determination of NO production. To determine the rate of appearance of citrulline and the rate of conversion to arginine a citrulline tracer is employed (Figure 3).

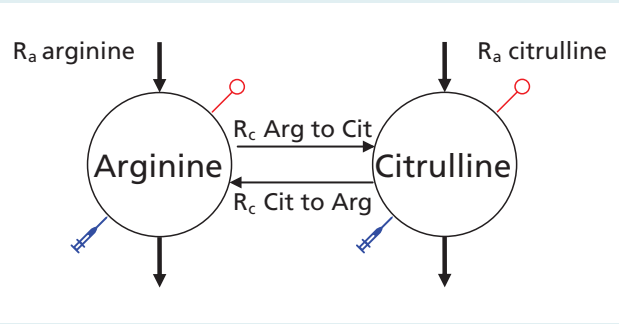


Figure 2. Arginine and citrulline rate of appearance (Ra) and rate of interconversions (Rc) model. Two different tracers are infused ( ) and the arginine and citrulline pool sampled ( ).

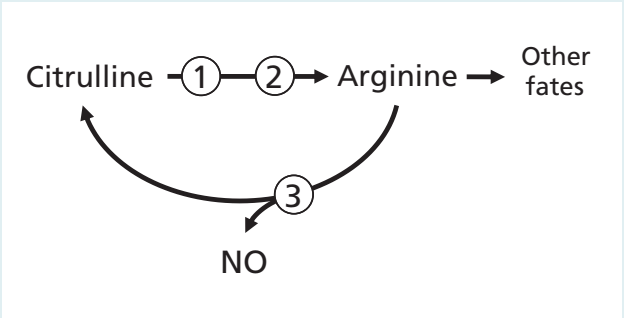


Figure 1. Arginine is synthesized from citrulline by action of argininosuccinate synthase (1) and argininosuccinate lyase (2). In turn, citrulline is a byproduct of the synthesis of NO by action of NO synthase (3).

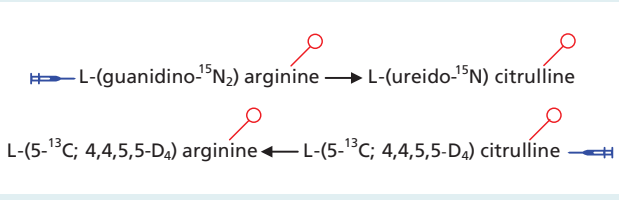


Figure 3. Tracer protocol for the determination of aginine and citrulline rate of appearance and rate of interconversions. After L-(guanido-$^{15}N_2$) arginine and L-(5-^{13}C; 4,4,5,5-D_4) citrulline (CDLM-7139) infusion ( ), the arginine and citrulline pools are sampled ( ) and analyzed for the isotopologues shown in the figure.

(continued)

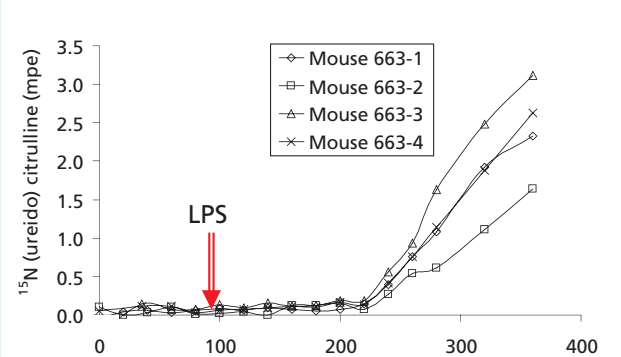


Figure 4. (Ureido-^{15}N) citrulline enrichment in mice challenged with endotoxin. The arrow denotes the time of endotoxin challenge.

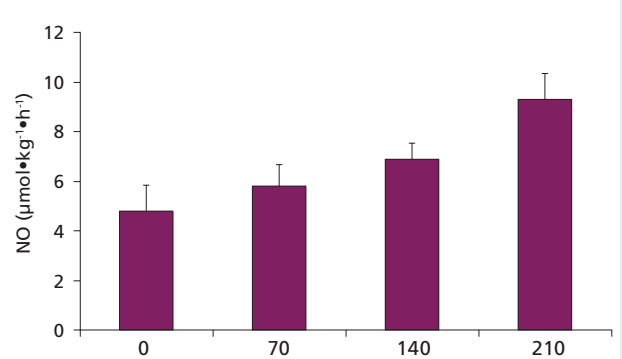


Figure 5. Nitric oxide production in mice supplemented with arginine four hours after endotoxin challenge. A linear relationship between arginine availability and NO response (R^2 = 0.57; P <0.0001) was observed.

The loss of the ureido nitrogen of citrulline during fragmentation in LC/MS/MS analysis results in a reduced natural (background) enrichment (~0.4 mp), and thus in the ability to reliably detect small enrichments above background (Figure 4). In addition, the derivatization of citrulline (*e.g.* dansylation) increases sensitivity and improves chromatography.

This isotopic approach has allowed us to study the NO time-dependent response after endotoxin (LPS) challenge and the effect of arginine supplementation on NO production during endotoxemia. Under basal conditions very little NO is produced as shown by the reduced (ureido-^{15}N) citrulline enrichment before LPS challenge (Figure 4). Following a ~2h lag period after endotoxin administration a dramatic increase in NO production can be detected. In another set of mice, the intravenous arginine supplementation in endotoxin-challenged mice resulted in a linear increase in NO production at 4h post LPS administration (Figure 5).

For over the past 15 years[1] these methods have proven to be useful in the determination of whole body nitric oxide production and *de novo* arginine production in various species. For an in depth discussion on the tracer methodology for the determination of NO see Van Eijk *et al.*[2] and for a comprehensive review on the human data, Siervo *et al.*, 2011.[3]

References

1. Castillo, L. **1996**. Whole body nitric oxide synthesis in healthy men determined from [^{15}N] arginine-to-[^{15}N] citrulline labeling. *Proc Natl Acad Sci USA, 93*, 11460-11465.
2. van Eijk, H.M.H.; Luiking, Y.C.; and Deutz, N.E.P. **2007**. Methods using stable isotopes to measure nitric oxide (NO) synthesis in the L-arginine/NO pathway in health and disease. *J. Chromatogr, B: Anal Technol Biomed Life Sci, 851*, 172-185.
3. Siervo, M. **2011**. Measurement of *in vivo* nitric oxide synthesis in humans using stable isotopic methods: A systematic review. *Free Radical Biol Med, 51*, 795-804.

Related Products

Catalog No.	Description
NLM-395	L-Arginine•HCl (guanido-$^{15}N_2$, 98%+)
NLM-6850	L-Citrulline (ureido-$^{15}N_1$, 98%)
CDLM-7139	L-Citrulline (5-^{13}C, 99%; 4,4,5,5-D_4, 95%)

Fatty Acid and Lipid Metabolism

Lipid metabolism refers to the metabolic processes involved in the synthesis and degradation of lipids in an organism. Fatty acid metabolism consists of the catabolic and anabolic processes that generate energy, primary metabolites and other key biological molecules from fatty acids. Combined with powerful mass spectrometry techniques, stable isotope tracers can be used to quantify the appearance and decomposition rates of lipids, fatty acids and glycerol in the body. Specifically, stable isotope tracers are often used to study fatty acid oxidation, glycerol kinetics and *de novo* lipogenesis, and lipolysis. Below is a list of stable isotope-labeled substrates that can be utilized to study fatty acid and lipid metabolism. **For a full list of substrates, please see the product listing starting on page 129.**

Related Products

Catalog No.	Description
DLM-9180	Docosanoic acid (22,22,22-D_3, 98%)
CLM-1397	Glycerol (2-^{13}C, 99%)
CLM-1857	Glycerol (1,3-$^{13}C_2$, 99%)
CLM-1510	Glycerol ($^{13}C_3$, 99%)
DLM-1229	Glycerol (1,1,2,3,3-D_5, 99%)
DLM-558	Glycerol (D_8, 99%)
CDLM-7745	Glycerol ($^{13}C_3$, 99%; D_8, 98%) CP 95%
DLM-8510	Hexacosanoic acid (12,12,13,13-D_4, 98%)
CLM-3960	Linoleic acid, ethyl ester (linoleate-U-$^{13}C_{18}$, 98%) CP 95%+
DLM-208	Myristic acid (D_{27}, 98%)
CLM-293	Octanoic acid (1-^{13}C, 99%)
CLM-3981	Octanoic acid ($^{13}C_8$, 99%)
CLM-3707	2-Octanoyl-1,3-distearin (octanoic-1-^{13}C, 99%)
CLM-2492	Oleic acid (methyl-^{13}C, 99%)
CLM-460	Oleic acid (U-$^{13}C_{18}$, 98%) CP 95%
DLM-1891	Oleic acid (D_{33}, 98%)
CLM-8856	Oleic acid, potassium salt (U-$^{13}C_{18}$, 98%) CP 95%
CLM-8763	Oleic acid, sodium salt (U-$^{13}C_{18}$, 98%)
CLM-150	Palmitic acid (1-^{13}C, 99%)
CLM-409	Palmitic acid (U-$^{13}C_{16}$, 98%)
DLM-215	Palmitic acid (D_{31}, 98%)
DLM-606	L-α-Phosphatidylcholine, dipalmitoyl (DPPC) (dipalmitoyl-D_{62}, 98%) CP 95%
CLM-1889	Potassium palmitate (1-^{13}C, 99%)
CLM-3943	Potassium palmitate (U-$^{13}C_{16}$, 98%+)
DLM-3773	Potassium palmitate (2,2-D_2, 97%)
CLM-441	Sodium bicarbonate (^{13}C, 99%) CP 97%+
CLM-174	Sodium palmitate (1-^{13}C, 99%)
CLM-6059	Sodium palmitate ($^{13}C_{16}$, 98%+)
DLM-379	Stearic acid (D_{35}, 98%)
DLM-9179	Tetracosanoic acid (9,9,10,10-D_4, 98%)
CLM-162	Trioctanoin (1,1,1-$^{13}C_3$, 99%)
CLM-163	Triolein (1,1,1-$^{13}C_3$, 99%)
CLM-164	Tripalmitin (1,1,1-$^{13}C_3$, 99%)

"Conventional cell-based TG (triglyceride) synthesis assays use radio-labeled glycerol or fatty acids to metabolically label TG molecules. The radio-labeled hydrophobic TG product is typically resolved by thin layer chromatography. However, LC/MS/MS is becoming the preferred method for quantitative lipid analysis due to the ease of automation, accuracy, sensitivity and the avoidance of radioactivity. We developed a high-throughput LC/MS/MS-based cellular assay for determining cellular DGAT activity using $^{13}C_3$-D_5-glycerol in lieu of radio-labeled glycerol."

Jenson Qi, PhD
Johnson and Johnson Pharmaceutical Research and Development, LLC, Spring House, PA

Reference

Excerpt from Qi, J., *et. al.* **2012**. The Use of Stable Isotope-Labeled Glycerol and Oleic Acid to Differentiate the Hepatic Functions of Diacylglycerol Acyltransferase-1 and -2. J. *Lipid Res, 53,* 1106-16.

Mixed Fatty Acids and Mixed Triglycerides

Catalog No.	Description
CLM-8455	Mixed fatty acids (U-^{13}C, 98%+)
DLM-8572	Mixed fatty acids (U-D, 98%)
CDLM-8376	Mixed fatty acids (U-^{13}C, 98%+; U-D, 97%+)
CLM-8381	Mixed fatty acids methyl esters (U-^{13}C, 98%+) (terminal ester unlabeled) CP 95%
DLM-2497	Mixed fatty acid methyl esters (U-D, 96-98%)
CLM-8373	Mixed triglycerides (U-^{13}C, 98%+)
DLM-8375	Mixed triglycerides (U-D, 97%+)

Composition of Mixed Fatty Acids

Palmitic acid:	35-55%
Palmitoleic acid:	10-15%
Oleic acid:	20-35%
Linoleic acid:	10-20%

Composition of labeled mixed fatty acids varies lot to lot.

Application Note 31

Tracing Lipid Disposition *in vivo* Using Stable Isotope-Labeled Fatty Acids and Mass Spectrometry

David G. McLaren, Steven J. Stout, Sheng-Ping Wang, Paul L. Miller, Kithsiri Herath, Vinit Shah, Ablatt Mahsut, Jose Castro-Perez, Douglas G. Johns, Michele Cleary, Stephen F. Previs & Thomas P. Roddy
Merck Research Laboratories
Merck & Co., Inc. Rahway, NJ 07065-0900 USA

Lipids are ubiquitous molecules which serve a variety of important biological functions, including energy storage (triglycerides), modulation of cellular membrane structure and function (phospholipids and cholesterol), intracellular signaling and hormonal regulation. Dysfunctions of lipid metabolism contribute to a variety of diseases including, among others, atherosclerosis, hypertriglyceridemia and type 2 diabetes. As such, understanding the synthesis, regulation and transport of lipids in the body is important to developing new and improved therapies for these diseases. Stable isotopes have been used to study several aspects of lipid metabolism including the synthesis and disposition of cholesterol,[1,2] phospholipids[3] and VLDL triglycerides.[4] In this application note, we highlight some of the advantages and experimental considerations for using stable isotope-labeled fatty acids as substrates to study lipid metabolism *in vivo* in mice.

Experimental Design

For experiments on lipid synthesis, C57BL6 mice were treated with a vehicle control or a systemic, small-molecule inhibitor of microsomal triglyceride transfer protein (MTP).[5] One hour later, the mice were administered 150 mg/kg of Oleic acid, potassium salt (U-$^{13}C_{18}$, 98%) CP 95% (CLM-8856) mixed with corn oil (Figure 2) or 20% TPGS (Figure 3). Blood samples were withdrawn at serial timepoints following tracer administration and processed to plasma. 10 µL of plasma were mixed with 90 µL of methanol containing heavy internal standards and further diluted with 300 µL of pentanol. The samples were centrifuged briefly to pellet insoluble proteins and 5-10 µL of the supernatant were analyzed by ultraperformance liquid chromatography interfaced with either a triple quadrupole (Waters Xevo TQ) or quadrupole-time of flight (Waters Synapt G2) mass spectrometer. For experiments on fatty acid oxidation, mice were administered a cocktail containing 150 mg/kg of three fatty acids – Palmitic acid, Stearic acid and Oleic acid mixed with intralipid. Experiments were conducted in which one of the fatty acids was perdeuterated Palmitic acid (D_{31}, 98%) (DLM-215), Stearic acid (D_{35}, 98%) (DLM-379), Oleic acid (D_{33}, 98%) (DLM-1891) and the remaining two were unlabeled, or in which all three fatty acids were perdeuterated. 10 µL of plasma were incubated with acetone at basic pH to exchange deuteriums in the plasma water with acetone. The deuterium enrichment in acetone was then measured by headspace gas chromatography interfaced with an isotope ratio mass spectrometer (Thermo Scientific). Further details on the methods employed in these experiments can be found in references.[6-8]

Data Analysis

Total plasma triglycerides were analyzed using a commercially available biochemical kit (Thermo Scientific). All other data were acquired and analyzed using the Waters or Thermo instrument software packages. Multiple reaction monitoring on a triple quadrupole mass spectrometer was used to trace the appearance of Oleic acid, potassium salt (U-$^{13}C_{18}$, 98%) CP 95% in selected triglycerides and cholesteryl ester in the blood. The concentration of [$^{13}C_{18}$] oleate-labeled lipids was determined from the peak area ratio of the analyte to its matched, heavy-labeled internal standard. Concentrations were plotted as a function of time, and the area under the curve was determined using the Graphpad Prism software package. Alternatively, data were manually mined from full scan MSe spectra obtained on the quadrupole-time of flight instrument. Selected ion chromatograms were extracted, and the relative abundance of each [$^{13}C_{18}$] oleate-labeled lipid was determined based on the peak area ratio to a class-specific internal standard.

Results

In particular, we have studied the Microsomal Triglyceride Transfer Protein (MTP), which facilitates the transfer of lipids onto growing lipoprotein particles in the gut and liver prior to their secretion into the systemic circulation (Figure 1). Inhibition of MTP prevents lipidation of these chylomicron and VLDL particles and thereby prevents lipids from entering the blood. The data presented in Figure 2 help to illustrate some of the advantages of using stable isotope tracers to study the effects of MTP inhibition on complex lipid metabolism. Panel (a) shows the effects of a single dose of the MTP inhibitor on total endogenous (*i.e.* unlabeled) plasma triglycerides following an oral bolus of corn oil. Although the effects of the MTP inhibitor can be quite clearly observed, the basal level of pre-existing, circulating triglycerides (30-50 mg/dL) establishes a lower limit on the window for measuring dynamic changes. Panel (b) shows data from the same experiment but for a specific triglyceride composed of palmitoyl, linoleoyl and [$^{13}C_{18}$] oleoyl fatty acids. Because this species does not exist in the system until after the tracer fatty acid is introduced there is no background level and the time zero sample is truly blank. Since

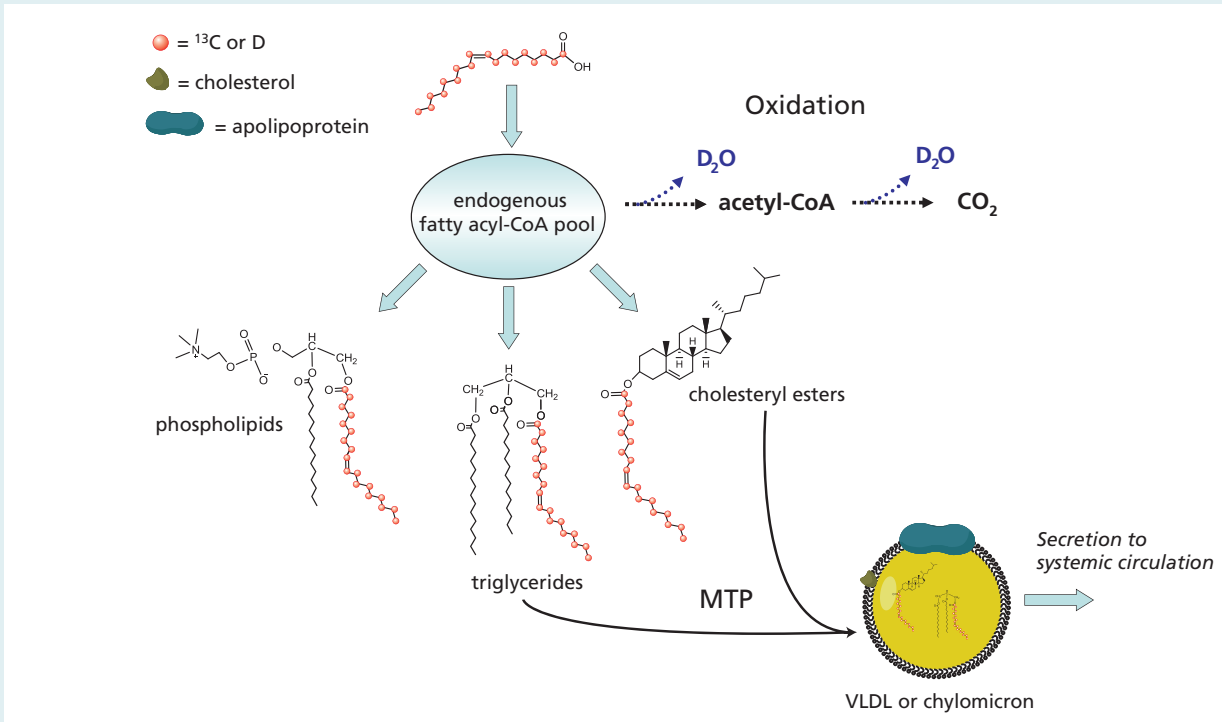


Figure 1. Schematic illustration showing the incorporation of [^{13}C] or [D] oleic acid into larger lipids. The isotope tracer enters the common fatty acid pool and is activated to a fatty acyl-CoA before being synthesized into phospholipid, triglyceride, cholesteryl ester or other lipids. In the liver and intestine, microsomal triglyceride transfer protein (MTP) aids in packaging triglycerides and cholesteryl esters into VLDL or chylomicron lipoprotein particles for secretion to the systemic circulation. Alternatively, [D] oleoyl-CoA can be oxidized to D_2O and CO_2.

complete inhibition of new triglycerides entering the system can now be feasibly measured, we achieve the theoretical maximum window for measuring dynamic changes in excursion due to the pharmacological intervention. Panel (c) compares the effects of the acute dose of the MTP inhibitor on circulating levels of cholesteryl oleate. The turnover of cholesteryl esters *in vivo* is relatively slow compared to that of triglycerides, and the single dose of the MTP compound given has no effect on the endogenous pool of this lipid. In stark contrast, by focusing the analysis on newly synthesized esters (*i.e.*, those tagged with the oleic acid tracer) we are able to observe a highly significant reduction in the amount of new lipid entering the system. Panel (d) illustrates that fatty acid oxidation can also be measured *in vivo* using a very similar experimental approach. In this experiment, we were interested in determining whether the nature of the fatty acid administered (palmitic acid vs. stearic acid vs. oleic acid) would affect the level of oxidation observed in normal mice. As the figure illustrates, comparable data on deuterium enrichment of plasma water was obtained regardless of which fatty acid was used as the tracer. Additionally, when a mixture containing perdeuterated forms of all three fatty acids was administered, the level of enrichment of plasma water was greater than what was observed in experiments when only a single fatty acid was labeled. In principal, experiments can also be designed in which lipid synthesis and fatty acid oxidation are measured simultaneously by simply using a [D]- rather than [^{13}C]-labeled fatty acid to measure synthesis.

Discussion

One principle of using isotope tracers to study biological processes is that the introduction of the isotope should not perturb the pathways under investigation. Simply put, tracers should be given in small enough amounts so as not to significantly alter the pools of the end products being traced. In order to achieve this, very sensitive analytical methods are required to enable measurement of the tracer enrichments. In the examples explored here, we have used [^{13}C]-labeled fatty acids as precursors for the synthesis of complex lipids (products). By employing ultraperformance liquid chromatography with mass spectrometric detection, we are able to measure very small amounts of [^{13}C]-labeled products in the presence of vastly greater endogenous (*i.e.* unlabeled) complex lipids.

Figure 3 (a) shows a total ion chromatogram of a mouse plasma lipid profile acquired following analysis by UPLC-qTOF-MS and includes lysophospholipids, phospholipids, diglycerides, ceramides, triglycerides and cholesteryl esters. Figure 3 (b) shows the mass spectrum of triglycerides eluting from the UPLC into the mass spectrometer between 8.5 to 10 minutes. As can be seen, a variety of triglycerides with different masses (based on permutations in fatty acid chain length and unsaturation) are present in this plasma extract. To take one example, the peak at m/z 902.8189 corresponds to triolein, a triglyceride containing

(continued)

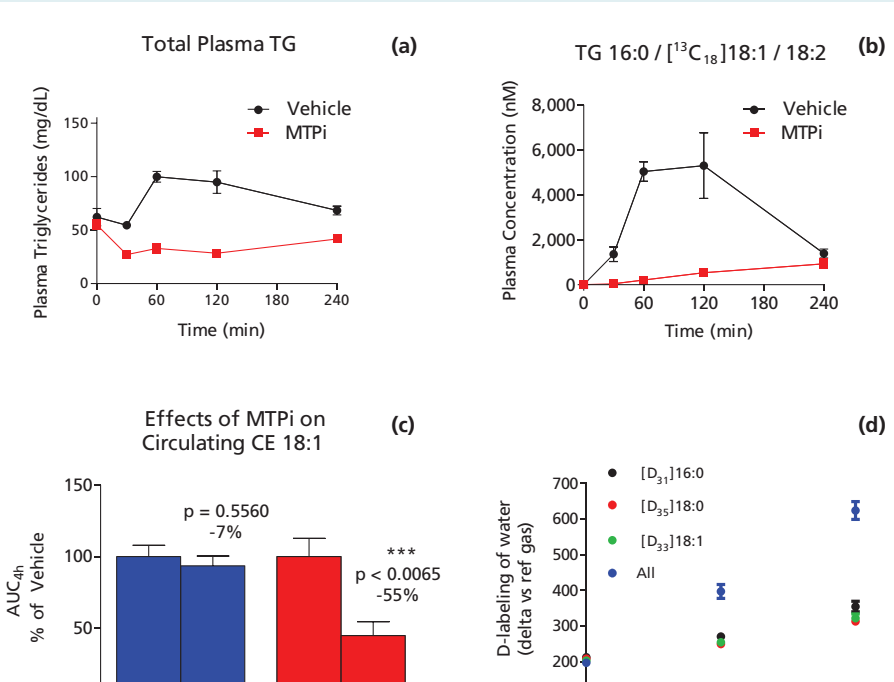


Figure 2. Measurement of lipid synthesis or fatty acid oxidation *in vivo* in mice. Panel (a) shows the influence of an MTP inhibitor on total plasma triglyceride levels in mice given an oral corn oil lipid challenge. Panel (b) shows the improvement in window for measuring the change in triglyceride excursion that can be gained by using a stable isotope-labeled fatty acid to distinguish newly synthesized lipids from endogenous background. Panel (c) illustrates the same advantage as applied to influences on levels of circulating cholesteryl ester over the 4h timecourse of the experiment. Panel (d) shows that oxidation of [D]-labeled fatty acids can be used to study oxidation by measuring the [D] enrichment in plasma water.

three equivalents of oleic acid. The mice in this experiment had been given a low, oral dose of Oleic acid, potassium salt (U-$^{13}C_{18}$, 98%) CP 95% (in the absence of any other naturally occurring fatty acids) prior to plasma being collected and processed. Panels (c) – (e) are enlargements of selected spectral features corresponding to various isotopomers of triolein. The peaks at m/z 920.8732, 938.9407 and 957.003 represent distinct molecules of triolein containing one, two or three equivalents of [$^{13}C_{18}$] oleate, respectively. The most intense [$^{13}C_{18}$] oleate-labeled isotopomer occurs at m/z 957.003 and is present at ~ 1/5 the concentration of the unlabeled endogenous triolein. In terms of totals, the sum of all [$^{13}C_{18}$] oleate-labeled triglycerides detected amounted to less than 1% of the concentration of total unlabeled (endogenous) triglyceride, illustrating the feasibility of performing these tracer studies without perturbing the natural biology.

There are a variety of practical benefits to adopting stable isotope tracer approaches for the study of lipid dynamics. One principle advantage is the ability to distinguish acute effects on dynamic lipid synthesis and disposition in the absence of steady-state effects. As illustrated in Figure 2(c), MTP inhibition does have an immediate impact on the appearance of intestinally derived, newly synthesized cholesteryl esters in the blood, though the total levels of the ester did not change over the timeframe of the experiment. These results obtained in mice are similar to what has been observed by others in humans. Although acute doses of this MTP inhibitor in humans was shown to lower VLDL cholesterol content, total and LDL cholesterol were not found to change over a 24-hour period.[5] Prolonged treatment, however, did result in changes in total and LDL cholesterol after three days of dosing. By using stable isotope tracers to distinguish newly synthesized from pre-existing cholesteryl esters, it is possible to get an indication of the effects of this compound in a much shorter period of time. Another related benefit of the tracer approach is the improvement in the window for measuring a dynamic change in lipid synthesis. By examining Figure 2(a) and (b), we can see that an approximately two-fold window is available for measuring changes in total plasma triglycerides. If this is the maximum change that can be observed, and with some knowledge of the inter-subject variability in these measurements, statistical power calculations can be carried out to determine the number of subjects that would be required to observe a specific %

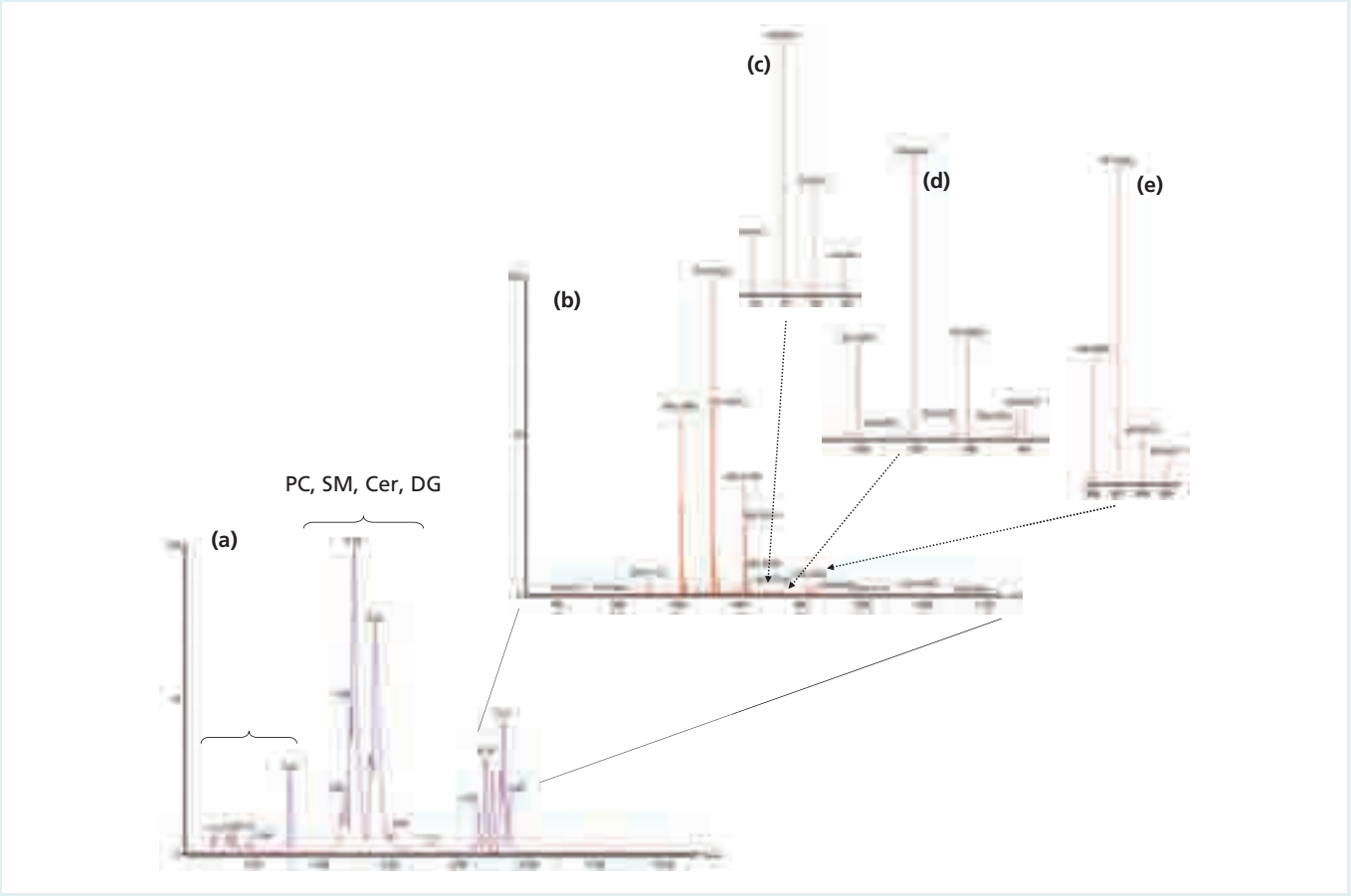


Figure 3. Untargeted analysis for the incorporation of Oleic acid, potassium salt (U-$^{13}C_{18}$, 98%) CP 95% into lipids in mouse plasma using ultra-performance liquid chromatography coupled to a quadrupole-time of flight mass spectrometer. Panel (a) shows the total ion chromatogram for the full lipid profile; panel (b) shows a portion of the mass spectrum from 8.5 to 10 minutes (where triglycerides and cholesteryl esters are known to elute); panels (c), (d) and (e) show expanded spectra for the incorporation of 1, 2 and 3 equivalents of Oleic acid, potassium salt (U-$^{13}C_{18}$, 98%) CP 95% into triolein, respectively. This figure highlights the detection of low levels of stable isotope-labeled fatty acids in the presence of much larger amounts of endogenous lipid.

change in plasma TG. In contrast, the tracer data provides an approximately seven-fold window for measuring this change. Based on this data set, power calculations suggest that the tracer methodology has the power to detect changes in new triglyceride synthesis using five-six times fewer subjects than traditional methods based on measuring changes in total triglycerides.

These examples help to highlight how the use of stable isotope tracers can have both practical and ethical benefits when conducting pharmaceutical research. By using stable isotope tracers to evaluate the effects of novel drug candidates in preclinical species, we can reduce the number of animals required to complete a given study. An extension of this is that we can also complete individual studies in less time, thereby making informed decisions more quickly. These approaches thus have the potential to reduce both the cost and time for development of new drugs, hopefully allowing novel treatments for diseases to be discovered and effective therapies to be advanced more rapidly for the patients that need them.

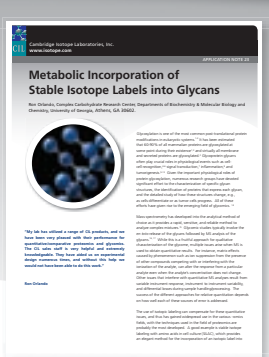
Related Products

Catalog No.	Description
CLM-8856	Oleic acid, potassium salt (U-$^{13}C_{18}$, 98%) CP 95%
DLM-1891	Oleic acid (D_{33}, 98%)
DLM-215	Palmitic acid (D_{31}, 98%)
DLM-379	Stearic acid (D_{35}, 98%)

(continued)

References

1. Di Buono, M.; Jones, P. ; Beaumier, L.; and Wykes, L. **2000**. Comparison of deuterium incorporation and mass isotopomer distribution analysis for measurement of human cholesterol biosynthesis. *J Lipid Res, 41*, 1516.
2. Jones, P.; Leitch, C.; Li, Z.; and Connor, W. **1993**. Human cholesterol synthesis measurement using deuterated water. Theoretical and procedural considerations. *Arterioscler, Thromb, Vasc Biol, 13*, 247.
3. DeLong, C.; Shen, Y.; Thomas, M.; and Cui, Z. **1999**. Molecular distinction of phosphatidylcholine synthesis between the CDP-choline pathway and phosphatidylethanolamine methylation pathway. *J Biol Chem, 274*, 29683.
4. Barrows, B.; and Parks, E. **2006**. Contributions of different fatty acid sources to very low-density lipoprotein-triacylglycerol in the fasted and fed states. *J. Clin Endocrinol Metab., 91*, 1446.
5. Chandler, C.; Wilder, D.; Pettini, J.; Savoy, Y.; Petras, S.; Chang, G.; Vincent, J.; and Harwood Jr., H. **2003**. CP-346086: an MTP inhibitor that lowers plasma cholesterol and triglycerides in experimental animals and in humans. *J Lipid Res, 44*, 1887.
6. Castro-Perez, J.; Kamphorst, J.; DeGroot, J.; Lafeber, F.; Goshawk, J.; Yu, K.; Shockcor, J.; Vreeken, R.; and Hankemeier, T. **2010**. Comprehensive LC- MSE Lipidomic Analysis using a Shotgun Approach and Its Application to Biomarker Detection and Identification in Osteoarthritis Patients. *J Proteome Res, 9*, 2377-2389.
7. McLaren, D.G.; He, T.; Wang, S.P.; Mendoza, V.; Rosa, R.; Gagen, K.; Bhat, G.; Herath, K.; Miller, P.L.; and Stribling, S.; Taggart, A.; Imbriglio, J.; Liu, J.; Chen, D.; Pinto, S.; Balkovec, J.M.; DeVita, R.J.; Marsh, D.J.; Castro-Perez, J.M.; Strack, A.; Johns, D.G.; Previs, S.F.; Hubbard, B.K.; Roddy, T.P. **2011**. The use of stable-isotopically labeled oleic acid to interrogate lipid assembly *in vivo*: assessing pharmacological effects in preclinical species. *J Lipid Res, 52,* 1150-1151.
8. Mahsut, A.; Wang, S.P.; McLaren, D.G.; Bhat, G.; Herath, K.; Miller, P.L.; Hubbard, B.K.; Johns, D.G.; Previs, S.F.; Roddy, T.P. **2011**. Headspace analyses of 2H labeling of acetone: Enabling studies of fatty acid oxidation *in vivo*. *Anal Biochem, 408*, 351-353.



Metabolic Incorporation of Stable Isotope Labels into Glycans

Ron Orlando, Complex Carbohydrate Research Center
Departments of Biochemistry & Molecular Biology and Chemistry
University of Georgia, Athens, GA 30602 USA

Glycosylation is one of the most common post-translational protein modifications in eukaryotic systems.[1-3] It has been estimated that 60-90% of all mammalian proteins are glycosylated at some point during their existence[1,3] and virtually all membrane and secreted proteins are glycosylated.[2] Glycoprotein glycans often play crucial roles in physiological events such as cell-cell recognition,[4-6] signal transduction,[7] inflammation,[8] and tumorigenesis.[9-13] Given the important physiological roles of protein glycosylation, numerous research groups have devoted significant effort to the characterization of specific glycan structures, the identification of proteins that express each glycan, and the detailed study of how these structures change, *e.g.*, as cells differentiate or as tumor cells progress. All of these efforts have given rise to the emerging field of glycomics.[14]

Mass spectrometry has developed into the analytical method of choice as it provides a rapid, sensitive and reliable method to analyze complex mixtures.[15] Glycomic studies typically involve the *en toto* release of the glycans followed by MS analysis of the glycans.[16, 17] While this is a fruitful approach for qualitative characterization of the glycome, multiple issues arise when MS is used to obtain quantitative results. For instance, matrix effects, caused by phenomenon such as ion suppression from the presence of other compounds competing with or interfering with the ionization of the analyte, can alter the response from a particular analyte even when the analyte's concentration does not change. Other issues that interfere with quantitative MS analyses result from variable instrument response, instrument-to-instrument variability and differential losses during sample handling/ processing. The success of the different approaches for relative quantitation depends on how well each of these sources of error is addressed.

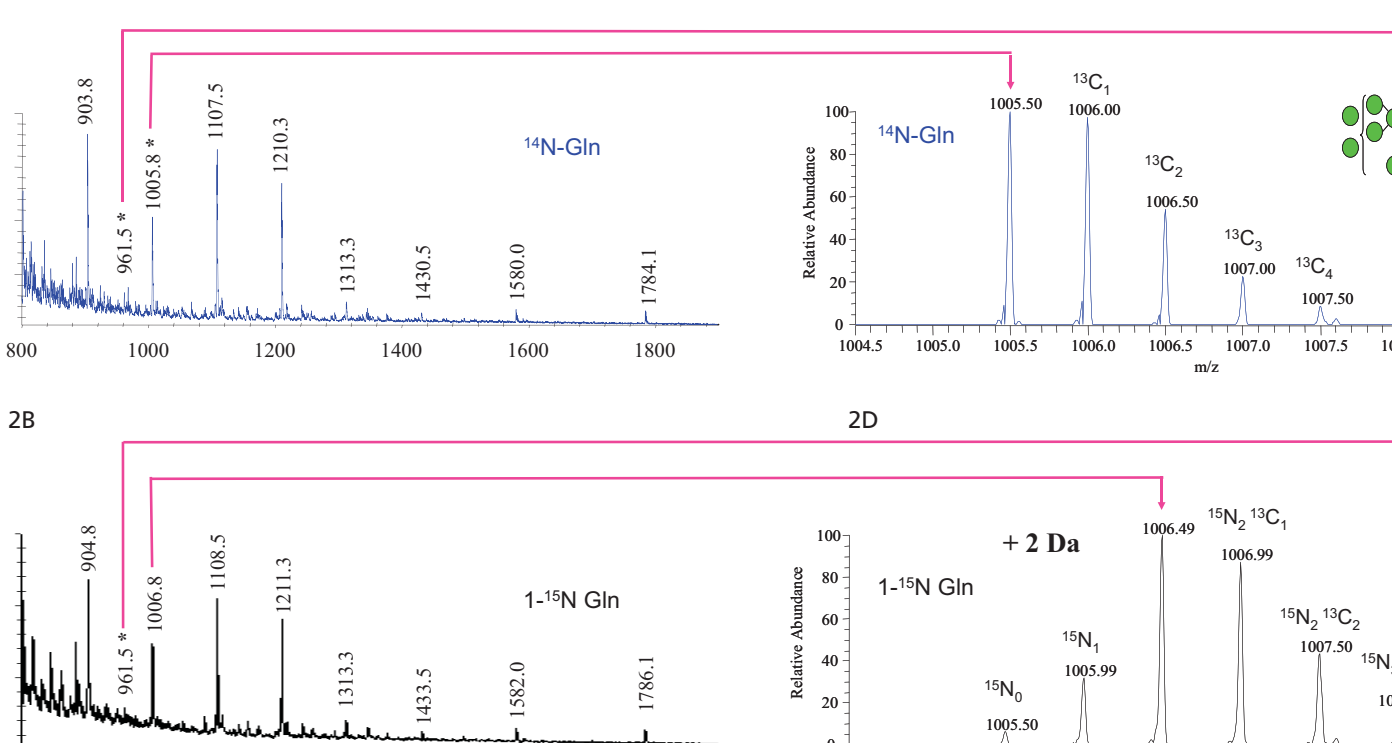
The use of isotopic labeling can compensate for these quantitative issues, and thus has gained widespread use in the various -omics fields, with the techniques used in the field of proteomics probably the most developed. A good example is stable isotope labeling with amino acids in cell culture (SILAC), which provides an elegant method for the incorporation of an isotopic label into proteins prior to MS-based proteomics.[18] In a SILAC experiment, two cell populations are grown in culture media that are identical except that one of them contains a "light"(natural abundance) and the other a "heavy"(isotopically enriched) form of a particular amino acid (unlabeled and L-Lysine•2HCl ($^{13}C_6$, 99%) (CLM-2247-H) and L-Arginine•HCl ($^{13}C_6$, 99%) (CLM-2265-H), for example). The isotopically heavy amino acids are supplied to cell cultures in place of the natural amino acids, and they become incorporated into all newly synthesized proteins. After multiple cell divisions, each instance of the particular amino acids is replaced by its "heavy" isotope-containing analog. An advantage of this approach is that the cells are mixed together immediately after cell lysis, thereby proteins from both cell types are subjected to the exact same experimental conditions during the sample handling, digestion, purification and separation steps. For this reason, SILAC is often considered the "gold standard" for comparitive quantitative proteomic analyses.[19]

In this note, an *in vivo* labeling strategy for glycomic studies is described which is analogous to the SILAC approach used for proteomics. This glycomic methodology is called IDAWG – Isotopic Detection of Aminosugars With Glutamine – and is based on the side-chain of glutamine being the sole nitrogen donor source in the biosynthesis of aminosugar nucleotides (see Figure 1). Thus, introduction of L-Glutamine (amide-^{15}N, 98%+), (NLM-557) into Gln-free media allows for all aminosugars, including GlcNAc, GalNAc and sialic acids, to become labeled with ^{15}N. This leads to the mass of *N*- and *O*-linked glycans, glycolipids and extracellular matrix polysaccharides to be increased by +1 Da per aminosugar. The utility of this approach is demonstrated by the analysis of *N*-linked glycans released from proteins of murine embryonic stem cells grown in both the preserve of light and amide-^{15}N-Gln. The success of these experiments leads us to predict that the IDAWG technology will be useful for a variety of future comparative glycomic studies in cell culture.

Results

The first and rate-limiting step in the hexosamine biosynthetic pathway (Figure 1) is the conversion of the glycolysis intermediate fructose-6-phosphate into glucosamine-6-phosphate.[20] The amino nitrogen introduced during this step is provided solely by the side-chain amide of Gln, which is converted to Glu.[21] Glucosamine-6-

(continued)



phosphate is the precursor of UDP-GlcNAc, which in turn leads to the other major aminosugar-containing sugar nucleotides, UDP-GalNAc and CMP-sialic acid[22, 23]. Thus, all GlcNAc, GalNAc and sialic acid-containing molecules are targets for isotopic labeling by supplementation of cell culture media with amide-^{15}N-Gln.

Initial experiments were performed to evaluate the possibility of using metabolic labeling as a method to incorporate a stable isotope into the glycans of cells grown in culture. In these experiments, R1 murine embryonic stem cells (mESCs) were grown using standard conditions. Cell culture media is typically supplied without glutamine, as this amino acid decomposes to glutamic acid and ammonia in aqueous solution. This formulation simplifies the IDAWG-labeling approach, because there is no need to specifically deplete the media of Gln. Either amide-^{15}N-Gln or unlabeled Gln was used to supplement the media at the standard concentration (2 mM). This straightforward substitution is the only alteration to the normal cell culturing procedure needed for IDAWG. In this example, murine ES cells were grown for three days with "heavy" or with "light" Gln, and then both *N*- and *O*-linked glycans were isolated from proteins for mass spectrometry-based analysis.

The incorporation of ^{15}N into the *N*-linked glycans of mESCs was investigated by comparing the FT-MS spectra of the permethylated *N*-linked glycans released from cells grown in either unlabeled-Gln or L-Glutamine (amide-^{15}N, 98%+) (Figures 2A and B). The abundant ions observed in these spectra correspond to high mannose glycans ($GlcNAc_2Man_{5\text{-}9}$) that are either singly or doubly charged. Comparison of these two spectra reveals that the glycan ions obtained from the cells grown in the "heavy" media are increased by 1 m/z unit for the doubly charged ions and 2 m/z units for the singly charged ions. This is the expected result provided that ^{15}N has been incorporated into the two core GlcNAcs contained in these glycans, which are the only nitrogens present in high mannose glycans.

This shift in glycan mass can be clearly seen upon closer inspection of the doubly charged molecular ion, $(M+2Na)^{2+}$, resulting from the $GlcNAc_2Man_7$ glycan, which appears at 1005.5 and 1006.5 m/z units from glycans grown in the ^{14}N and amide -^{15}N, respectively (Figure 2C and D). In these spectra, the most intense ion appears at the monoisotopic mass calculated with either ^{14}N or two ^{15}N, demonstrating that the majority of this glycan has ^{15}N incorporated into both possible sites. Ions are present that correspond to under labeling, *i.e.*, the incorporation of zero and one, ^{15}N, however the dominant species is fully labeled.

Larger *N*-linked glycans are similarly increased in mass by the incorporation of one ^{15}N per GlcNAc residue. This can be observed by expanding the spectra shown in Figure 2A and 2B so that only the region from 960.5 to 964.1 is observed (Figure 2E and F, for the glycans released from cells grown in ^{14}N and amide -^{15}N Gln media, respectively). This region shows the molecular

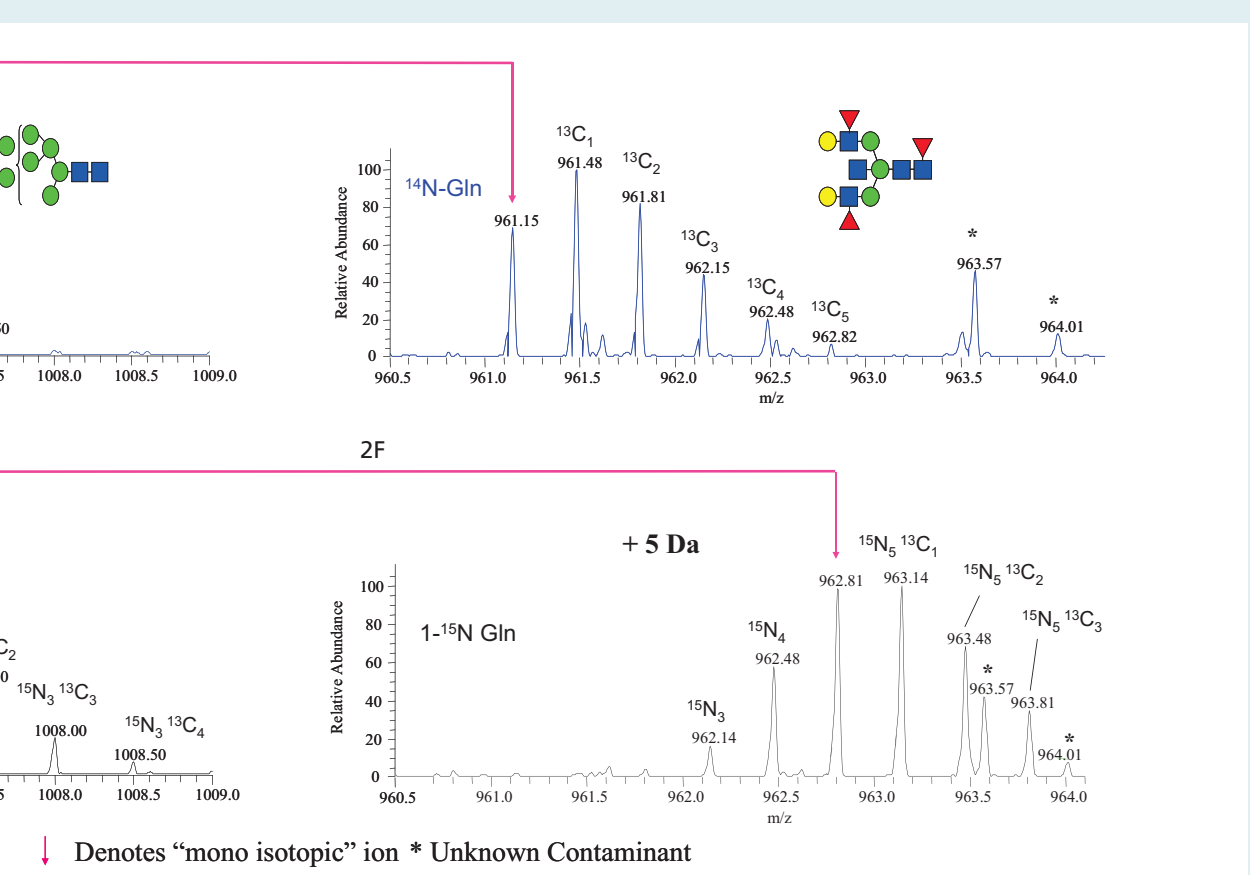


Figure 2. Isotopically labeled *N*-linked Glycans. Full FT-MS spectra from 850-2000 m/z of the permethylated *N*-linked glycans released from cells grown in either unlabeled-Gln(2A) or amide-^{15}N-Gln (2B). Expanded regions of the spectra showing the expected 2 Da mass shift from ^{15}N incorporation into the two core GlcNAc residues of the GlcNAc2Man7 glycan (2C,2D), and the 5 Da increase corresponding to ^{15}N incorporation into the five amniosugars of the $GlcNAc_5Man_3Gal_2Fuc_3$ glycan (2E,2F).

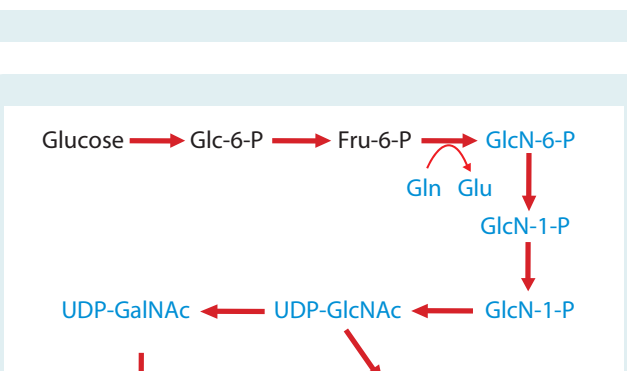


Figure 1. The hexosamine biosynthetic pathway demonstrating that the side-chain of glutamine is the sole donor source of nitrogen for amino sugars in the production of sugar nucleotides, which allows the introduction of an ^{15}N isotopic tag into all aminosugars, including GlcNAc, GalNAc and sialic acids. Species containing ^{15}N are indicated by blue type.

ion, $(M+3Na)^{3+}$, for a complex glycan with a composition of $GlcNAc_5Man_3Gal_2Fuc_3$. As predicted, the monoisotopic ion from this glycan is increased by 5 Da when it was obtained from cells grown in the amide-^{15}N Gln. In addition, the fully labeled glycan is the dominant species, although labeling is not complete as ions are observed from under labeling.

Calculations performed on the ratios of the intensities of the different isotopes in the ^{15}N spectrum indicate a 98% incorporation of ^{15}N into this glycan, which happens to equal the extent of ^{15}N in the glutamine used for this experiment. Similar calculations on the isotopic distributions from other glycans indicate that their ^{15}N incorporation ranged from 96-98%. Combined, these examples demonstrate that ^{15}N from L-Glutamine (amide-^{15}N, 98%+) becomes extensively incorporated into the GlcNAc residues of *N*-linked glycans, suggesting that this is potentially a useful strategy for introducing a stable isotope into glycans.

Discussion

Metabolic labeling of glycans provides many new opportunities for assessing the dynamics of glycan turnover during the course of any cellular behavior that can be induced or sustained in culture. For example, labeling cells with L-Glutamine (amide-^{15}N, 98%+) and then replacing the media supplement with "light" Gln will allow the determination of the half-life of any aminosugar-containing glycans. Previously, glycan turnover studies required incorporation of radioactive monosaccharide and extensive subsequent fractionation to identify specific changes in glycan expression. Generally, these radiotracer techniques allowed for very sensitive detection of glycan classes, but lacked the resolution to follow individual glycan structures or subsets of biosynthetically related species. The stable isotope incorporation method reported

(continued)

here merges the analytic advantages of high-resolution mass spectrometry to the biological necessity of understanding the dynamics of glycan turnover. Thus, IDAWG appears to be a powerful quantitative tool for exploring the biological role of glycans, glycoproteins and glycolipids in cell culture systems.

Acknowledgements

This work was funded by the National Institutes of Health/NCRR-funded Integrated Technology Resource for Biomedical Glycomics (P41 RR018502) and the National Institutes of Health/NCRR-funded Research Resource for Integrated Glycotechnology (P41 RR005351).

Related Products

Catalog No.	Description
CLM-2265-H	L-Arginine•HCl ($^{13}C_6$, 99%)
NLM-557	L-Glutamine (amide-^{15}N, 98%+)
CLM-2247-H	L-Lysine•2HCl ($^{13}C_6$, 99%)

References

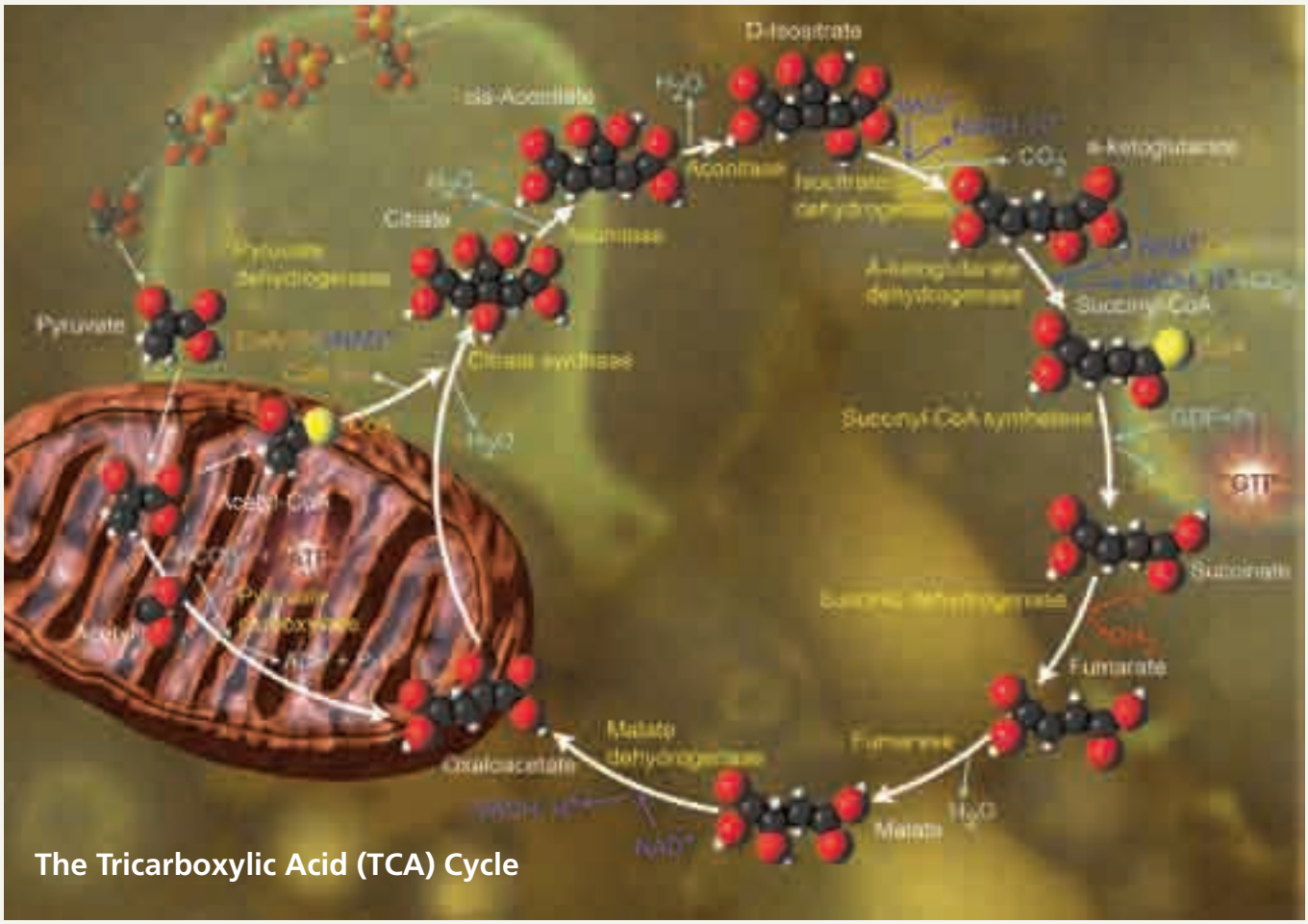
1. Varki, A. **1993**. Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology, 3*, 97-130.
2. Krueger, K.E.; Srivastava, S. **2006**. Posttranslational Protein Modifications: Current Implications for Cancer Detection, Prevention, and Therapeutics. *Molecular & Cellular Proteomics, 5*, 1799.
3. Apweiler, R.; Hermjakob, H.; Sharon, N. **1999**. On the frequency of protein glycosylation, as deduced from analysis of the SWISS-PROT database. *Biochim Biophy Acta, 1473*, 4-8.
4. Drickamer, K.; Taylor, M.E. **1998**. Evolving views of protein glycosylation. *Trends Biochem Sci, 23,* 321-324.
5. Dwek, R.A. **1995**. Glycobiology: more functions for oligosaccharides. *Science, 269*, 1234-5.
6. Lis, H.; Sharon, N. **1993**. Protein glycosylation. Structural and functional aspects. *FEBS Journal, 218*, 1-27.
7. Haltiwanger, R.S. **2002**. Regulation of signal transduction pathways in development by glycosylation. *Curr Opin Struct Biol, 12*, 593-8.
8. Lowe, J.B. **2003**. Glycan-dependent leukocyte adhesion and recruitment in inflammation. *Curr Opin Cell Biol, 15*(5), 531-8.
9. Fukuda, M. **1996**. Possible roles of tumor-associated carbohydrate antigens. *Cancer Res, 56*, 2237-44.
10. Hakomori, S. **1989**. Aberrant glycosylation in tumors and tumor-associated carbohydrate antigens. *Adv Cancer Res, 52,* 257-331.
11. Lowe, J.B.; Marth, J.D. **2003**. A genetic approach to Mammalian glycan function. *Annu Rev Biochem, 72,* 643-91.
12. Muramatsu, T. **1993**. Carbohydrate signals in metastasis and prognosis of human carcinomas. *Glycobiology, 3*, 291-6.
13. Olden, K. **1993**. Adhesion molecules and inhibitors of glycosylation in cancer. *Semin Cancer Biol, 4*, 269-76.
14. Lubner, G.C. **2003**. Glycomics: an innovative branch of science. *Boll Chim Farm, 142*, 50.
15. Zaia, J. **2004**. Mass spectrometry of oligosaccharides. *Mass Spectrometry Reviews, 23*, 161-227.
16. North, S.J.; Koles, K.; Hembd, C.; Morris, H.R.; Dell, A.; Panin, V.M.; Haslam, S.M. **2006**. Glycomic studies of Drosophila melanogaster embryos. *Glycoconjugate Journal, 23*, 345-354.
17. Jang-Lee, J.; North, S.J.; Sutton-Smith, M.; Goldberg, D.; Panico, M.; Morris, H.; Haslam, S.; Dell, A. **2006**. Glycomic profiling of cells and tissues by mass spectrometry: fingerprinting and sequencing methodologies. *Methods Enzymol., 415,* 59-86.
18. Ong, S.E.; Blagoev, B.; Kratchmarova, I.; Kristensen, D.B.; Steen, H.; Pandey, A.; Mann, M. **2002**. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Mol Cell Proteomics, 1*, 376-86.
19. Ong, S.E.; Foster, L.J.; Mann, M. **2003**. Mass spectrometric-based approaches in quantitative proteomics. *Methods, 29*(2), 124-30.
20. McClain, D.A. **2002**. Hexosamines as mediators of nutrient sensing and regulation in diabetes. *J Diabetes Complications, 16*, 72-80.
21. Yki-Jarvinen, H.; Vogt, C.; Iozzo, P.; Pipek, R.; Daniels, M.C.; Virkamaki, A.; Makimattila, S.; Mandarino, L.; DeFronzo, R.A.; McClain, D.; Gottschalk, W.K. **1997**. UDP-*N*-acetylglucosamine transferase and glutamine: fructose 6-phosphate amidotransferase activities in insulin-sensitive tissues. *Diabetologia, 40*, 76-81.
22. Kean, E.L.; Munster-Kuhnel, A.K.; Gerardy-Schahn, R. **2004**. CMP-sialic acid synthetase of the nucleus. *Biochim Biophys Acta, 1673*, 56-65.
23. Thoden, J.B.; Wohlers, T.M.; Fridovich-Keil, J.L.; Holden, H.M. **2001**. Human UDP-galactose 4-epimerase. Accommodation of UDP-*N*-acetylglucosamine within the active site. *J Biol Chem, 276*, 15131-6.

Investigator Spotlight

Cellular Metabolism and Metabolomics

Joshua D. Rabinowitz, PhD
Department of Chemistry, Princeton University
Princeton, NJ 08544 USA

Isotope tracers are essential tools for monitoring metabolic pathway activity, *i.e.* flux. To quantitate flux in central carbon metabolism in cultured mammalian cells, D-Glucose ($^{13}C_6$, 99%) (CLM-1396) or L-Glutamine ($^{13}C_5$, 99%) (CLM-1822) is added to media lacking these principal nutrients. Cells are grown in the labeled media, and metabolite labeling is measured by GC/MS or LC/MS. Glucose has traditionally been considered to be the primary carbon source for many cell types, especially cancer cells. Recent studies show, however, the glutamine often plays a predominant role in feeding the tricarboxylic acid (TCA) cycle. The extent of contribution of glutamine to TCA cycle four-carbon units can be measured based on malate and asparate labeling, and to two-carbon units based on acetyl-CoA and fatty acid labeling. Citrate is a particularly informative molecule, because it reflects both two- and four-carbon units of the TCA cycle. With modern instrumentation, it is possible to measure in parallel the isotope labeling of all of these species, and dozens more, enabling systems-level flux quantitation. These methods can translate also to the *in vivo* setting, with mice or patients infused with labeled nutrients prior to resection of a tumor or other tissue specimen. The importance of metabolism in both bioengineering and disease pathophysiology is leading to wide application of these methods across the biochemical sciences.



The Tricarboxylic Acid (TCA) Cycle

Cellular Metabolism and Metabolomics

Related Products

Catalog No.	Description
CNLM-534-H	L-Alanine ($^{13}C_3$, 99%; ^{15}N, 99%)
NLM-467	Ammonium chloride (^{15}N, 99%)
NLM-713	Ammonium sulfate ($^{15}N_2$, 99%)
CLM-2265-H	L-Arginine•HCl ($^{13}C_6$, 99%)
CNLM-539-H	L-Arginine•HCl ($^{13}C_6$, 99%; $^{15}N_4$, 99%)
DLM-546	L-Aspartic acid (2,3,3-D_3, 97-98%)
CLM-9021	Citric acid ($^{13}C_6$, 99%) CP 97%
CLM-147	Citric acid (2,3,4-$^{13}C_3$, 99%)
DLM-3487	Citric acid (2,2,4,4-D_4, 98%)
CLM-1824	2-Deoxy-D-Glucose (1-^{13}C, 99%)
CLM-2122	2-Deoxy-D-Glucose (6-^{13}C, 99%)
DLM-4	Deuterium oxide (D, 99.9%)
CLM-1553	D-Fructose (U-$^{13}C_6$, 99%)
CLM-1529	Fumaric acid ($^{13}C_4$, 99%)
DLM-1539	Fumaric acid (2,3-D_2, 98%)
CDLM-6062	Fumaric acid (1-^{13}C, 99%; 2,3-D_2, 98%)
CDLM-8473	Fumaric acid (1,4-$^{13}C_2$, 99%; 2,3-D_2, 98%)
CLM-744	D-Galactose (1-^{13}C, 99%)
CLM-1570	D-Galactose (U-$^{13}C_6$, 99%)
CLM-420	D-Glucose (1-^{13}C, 98-99%)
CLM-746	D-Glucose (2-^{13}C, 99%)
CLM-1393	D-Glucose (3-^{13}C, 99%)
CLM-1394	D-Glucose (4-^{13}C, 99%)
CLM-1395	D-Glucose (5-^{13}C, 98%)
CLM-481	D-Glucose (6-^{13}C, 99%)
CLM-504	D-Glucose (1,2-$^{13}C_2$, 99%)
CLM-2717	D-Glucose (1-^{13}C, 99%; 6-^{13}C, 97%+)
CLM-8942	D-Glucose (2,3-$^{13}C_2$, 99%)
CLM-6750	D-Glucose (3,4-$^{13}C_2$, 99%)
CLM-8770	D-Glucose (4,5,6-$^{13}C_3$, 98%)
CLM-1396	D-Glucose (U-$^{13}C_6$, 99%)
DLM-1150	D-Glucose (1-D, 98%)
DLM-1271	D-Glucose (2-D, 98%)
DLM-349	D-Glucose (6,6-D_2, 99%)
DLM-2062	D-Glucose (1,2,3,4,5,6,6-D_7, 98%)
CDLM-3813	D-Glucose (U-$^{13}C_6$, 99%; 1,2,3,4,5,6,6-D_7, 97-98%)
CLM-1510	Glycerol ($^{13}C_3$, 99%)

Catalog No.	Description
CLM-422	Glycine (1-^{13}C, 99%)
CLM-136	Glycine (2-^{13}C, 99%)
CLM-1017	Glycine (1,2-$^{13}C_2$, 97-99%)
CLM-3612	L-Glutamine (1-^{13}C, 99%)
CLM-1166	L-Glutamine (5-^{13}C, 99%)
CLM-2001	L-Glutamine (1,2-^{13}C, 99%)
CLM-1822-H	L-Glutamine ($^{13}C_5$, 99%)
DLM-1826	L-Glutamine (2,3,3,4,4-D_5, 97%)
NLM-1016	L-Glutamine (α-^{15}N, 98%)
NLM-557	L-Glutamine (amide-^{15}N, 98%)
NLM-1328	L-Glutamine ($^{15}N_2$, 98%)
NLM-4264	Inosine (U-$^{15}N_4$, 95%+)
CLM-2248-H	L-Isoleucine ($^{13}C_6$, 99%)
CLM-2411	α-Ketoglutaric acid (U-$^{13}C_5$, 99%)
CLM-4442	α-Ketoglutaric acid, disodium salt (1,2,3,4-$^{13}C_4$, 99%) CP 97%
CLM-2262-H	L-Leucine ($^{13}C_6$, 99%)
CLM-2247-H	L-Lysine•2HCl ($^{13}C_6$,99%)
CNLM-291-H	L-Lysine•2HCl ($^{13}C_6$, 99%; $^{15}N_2$, 99%)
DLM-1129	Maleic acid (2,3-D_2, 98%)
CLM-310	Maleic anhydride (1,4-$^{13}C_2$, 99%)
CLM-312	Maleic anhydride (2,3-$^{13}C_2$, 99%)
CLM-6019	Maleic anhydride ($^{13}C_4$, 99%)
CLM-359	Methanol (^{13}C, 99%)
CLM-893-H	L-Methionine ($^{13}C_5$, 99%)
DLM-651	Methyl formate (formyl-D, 99%)
DLM-6883	Nicotinamide (D_4, 98%)
CLM-3981	Octanoic acid ($^{13}C_8$, 99%)
CLM-460	Oleic acid (U-$^{13}C_{18}$, 98%) CP 95%
NLM-1048	Orotic acid:H_2O (1,3-$^{15}N_2$, 98%+)
CLM-409	Palmitic acid (U-$^{13}C_{16}$, 98%)
DLM-372	L-Phenylalanine (D_8, 98%)
CLM-3551	Potassium phosphoenol pyruvate (2-^{13}C, 99%)
CLM-2723	Potassium phosphoenol pyruvate (3-^{13}C, 99%)
CLM-3398	Potassium phosphoenol pyruvate (2,3-$^{13}C_2$, 99%)
CLM-8077	Pyruvic acid (1-^{13}C, 99%)
CLM-8849	Pyruvic acid (2-^{13}C, 99%) CP 95%

Cellular Metabolism and Metabolomics

Related Products *(continued)*

Catalog No.	Description
CLM-1573	L-Serine (1-^{13}C, 99%)
CLM-1572	L-Serine (3-^{13}C, 99%)
CLM-1574-H	L-Serine ($^{13}C_3$, 99%)
DLM-582	L-Serine (2,3,3-D_3, 98%)
CLM-1256	Sodium butyrate (1-^{13}C, 99%)
CLM-583	Sodium formate (^{13}C, 99%)
CLM-1577	Sodium L-lactate (1-^{13}C, 99%) (20% w/w in H_2O)
CLM-1578	Sodium L-lactate (3-^{13}C, 98%) (20% w/w in H_2O)
CLM-1579	Sodium L-lactate ($^{13}C_3$, 98%) (20% w/w in H_2O)
DLM-3317	Sodium L-lactate (3,3,3-D_3, 98%) (20% w/w in H_2O)
CLM-1082	Sodium pyruvate (1-^{13}C, 99%)
CLM-1580	Sodium pyruvate (2-^{13}C, 99%)
CLM-1575	Sodium pyruvate (3-^{13}C, 99%)
CLM-3507	Sodium pyruvate (2,3-$^{13}C_2$, 99%)
CLM-2440	Sodium pyruvate ($^{13}C_3$, 99%)
DLM-7311	Stearoyl coenzyme A (steroyl-methyl-D_3, 98%) CP 90%

Catalog No.	Description
CLM-1084	Succinic acid (1,4-$^{13}C_2$, 99%)
CLM-1199	Succinic acid (2,3-$^{13}C_2$, 99%)
CLM-1571	Succinic acid ($^{13}C_4$, 99%)
DLM-584	Succinic acid (D_4, 98%)
DLM-831	Succinic acid (D_6, 98%)
CDLM-7754	Succinic acid ($^{13}C_4$, 99%; 2,2,3,3-D_4, 98%)
DLM-2307	Succinic acid, disodium salt (D_4, 75%)
NLM-637	Uracil (1,3-$^{15}N_2$, 98%)
CLM-2249-H	L-Valine ($^{13}C_5$, 99%)
DLM-488	L-Valine (D_8, 98%)
CLM-1140	D-Xylose (1-^{13}C, 99%)
CLM-1524	D-Xylose (2-^{13}C, 99%)
CLM-8593	D-Xylose (3-^{13}C, 99%)
CLM-9083	D-Xylose (4-^{13}C, 99%)
CLM-1219	D-Xylose (5-^{13}C, 99%)
CLM-2456	D-Xylose (1,2-$^{13}C_2$, 99%)

"I have been extremely happy with all products and services that we've obtained from CIL. All stable isotope reagents have exceeded our expectations. We've also obtained custom services from CIL to complete some very challenging studies, and have found CIL flexible and willing to work with us to achieve our goals. In short, I have nothing but positive things to say about our experience with CIL and their products."

Matthew Steinhauser, MD
Brigham and Women's Hospital
Harvard Medical School

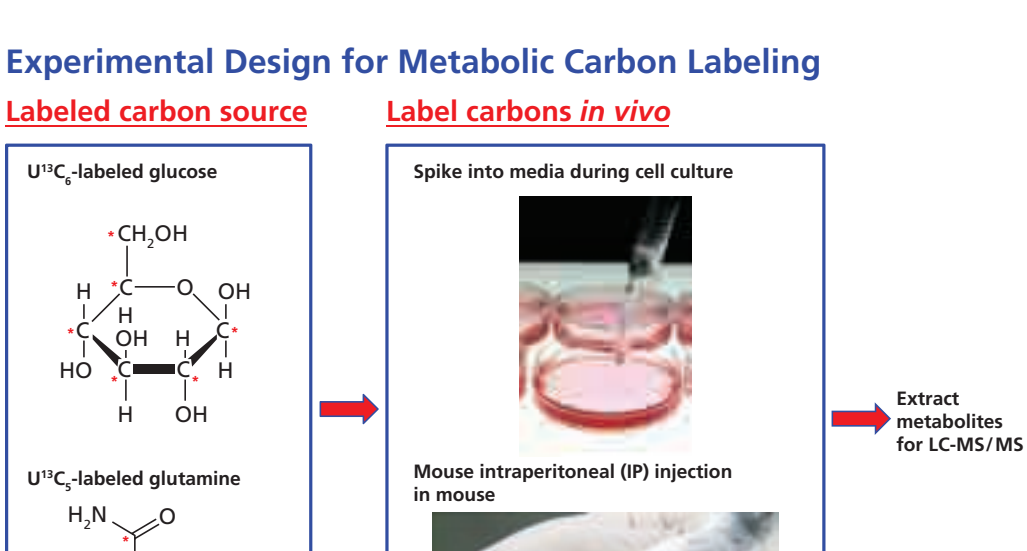
Fluxing Through Cancer: Tracking the Fate of ^{13}C-Labeled Energy Sources Glucose and Glutamine in Cancer Cells and Mouse Tumors

Costas A. Lyssiotis,[1,2] Susanne B. Breitkopf,[1,2] Min Yuan,[1] Gary Bellinger,[1] John M. Asara[1,2]
1. Beth Israel Deaconess Medical Center, Boston, MA 02215 USA
2. Harvard Medical School, Boston, MA 02115 USA

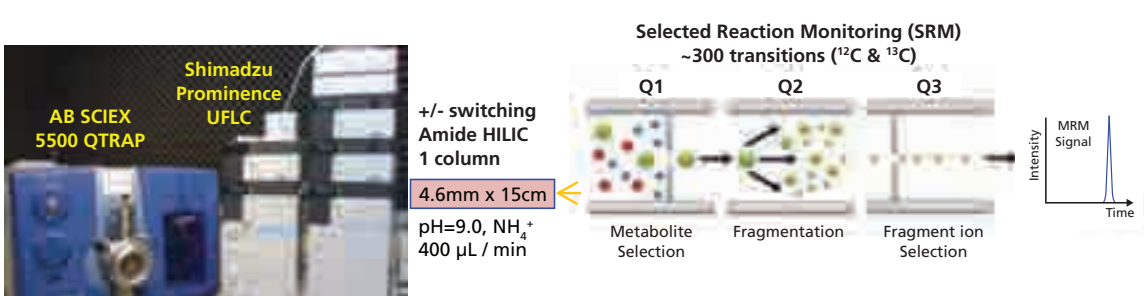
Abstract

Glucose and glutamine provide the primary energy sources for cell growth and proliferation. To study metabolic reprogramming, we used D-Glucose (U-$^{13}C_6$, 99%) (CLM-1396) and L-Glutamine ($^{13}C_5$, 99%) (CLM-1822-H) to target and track the diversion of these molecules into several metabolic pathways, including glycolysis, the TCA cycle, the pentose phosphate pathway, the metabolism of amino acids and nucleotides, *etc.* in both cell lines and mouse tumors. We use a positive/negative ion polarity switching single column SRM experiment during a 15-minute acquisition. For *in vivo* labeling experiments, D-Glucose (U-$^{13}C_6$, 99%) or L-Glutamine ($^{13}C_5$, 99%) solutions were delivered to tumors via intraperitoneal injection (IP) or jugular delivery and compared. Metabolites were extracted from cells or tumor tissues using 80% methanol. Metabolomics were performed on a AB/SCIEX 5500 QTRAP in SRM mode using amide XBridge HILIC chromatography with Q1/Q3 transitions for both the unlabeled and ^{13}C-labeled metabolites with separate methods for glucose and glutamine.

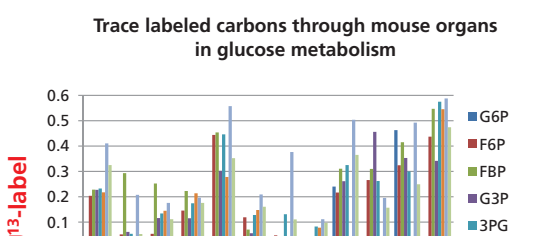
The platform targets more than 150 labeled metabolites (>250 unlabeled metabolites). Peaks were integrated using MultiQuant software and data analyzed using in-house developed tools, as well as MetaboAnalyst, MarkerView, *etc.* Cell line experiments were performed in biological triplicates and assays were derived from various cancers, including multiple myeloma and pancreatic cancer that had mutations or perturbations in a number of the genes known to affect cancer metabolism. The quantitative data from *in vivo* mouse models show the specific pathways where ^{13}C-labeled carbons from glucose or glutamine trace through metabolism providing valuable information regarding the defective and amplified metabolic pathways and could aid in the selection of therapeutic molecules that interfere with such pathways.



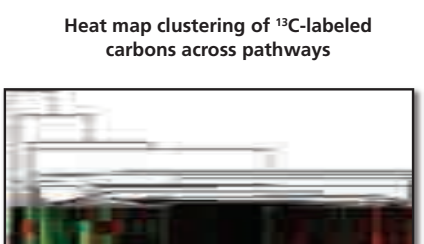
Targeted LC-MS/MS Platform for Metabolic Carbon Labeling



Trace labeled carbons through mouse organs in glucose metabolism

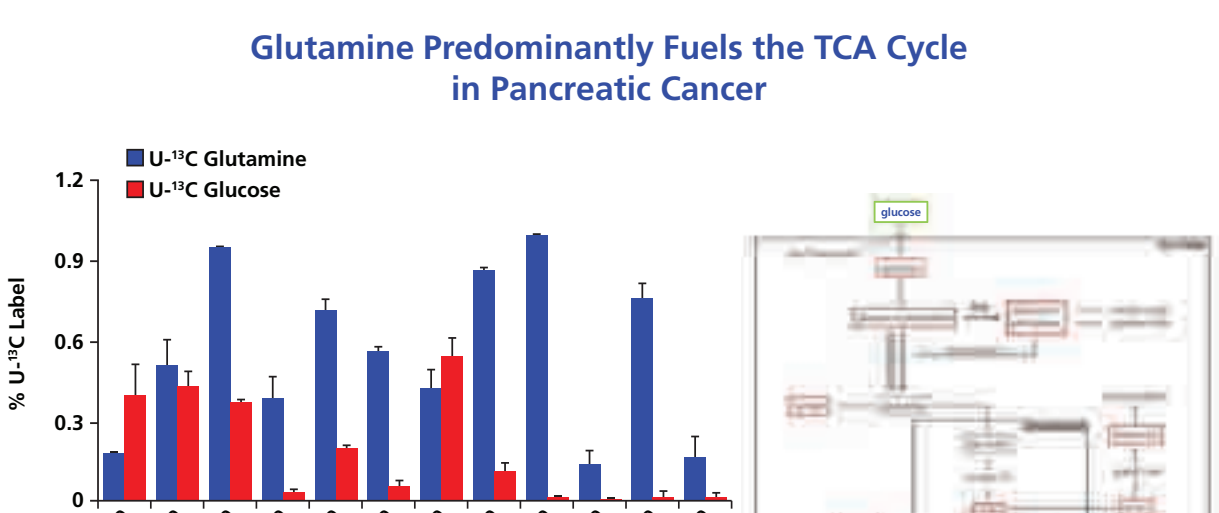


Heat map clustering of ^{13}C-labeled carbons across pathways



Yuan, M. *et. al.* **2012**. *Nat Protoc, 7*, 872-81.

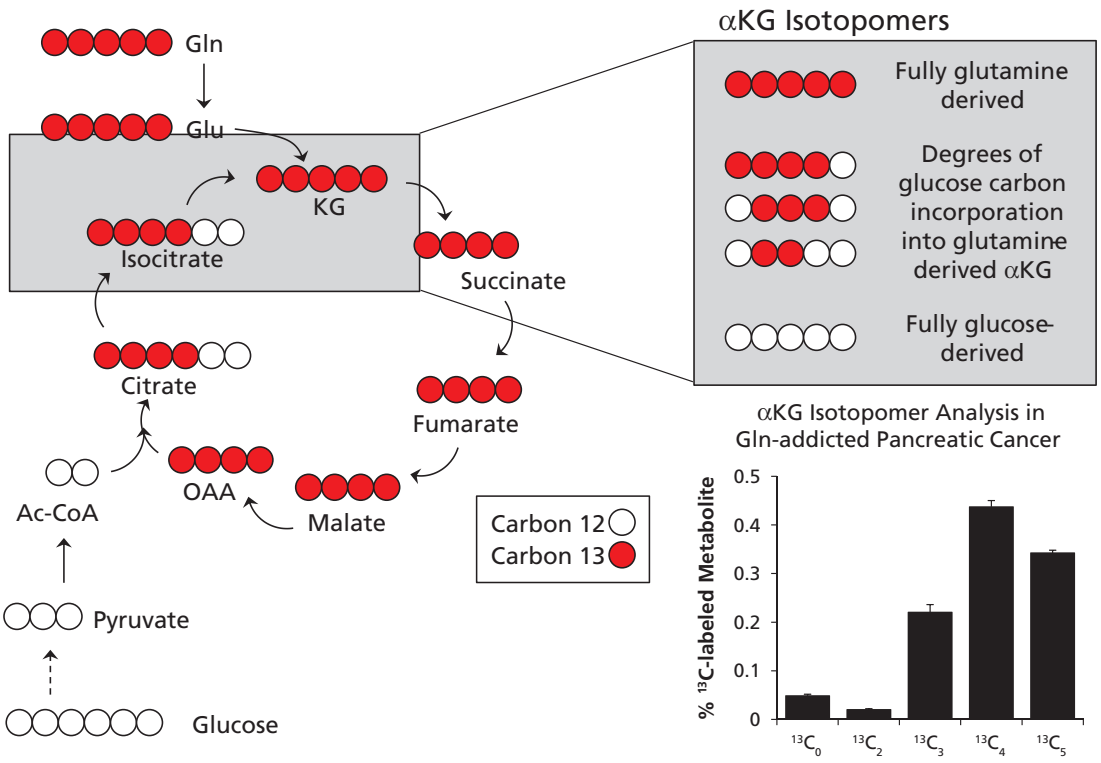
Glutamine Predominantly Fuels the TCA Cycle in Pancreatic Cancer



Ying, H. *et. al.* **2012**. *Cell, 149*, 656-70.

(continued)

Tracking Glutamine Metabolism by Isotope-Labeled Carbon



Son, J. *et. al.* **2013**. *Nature, 496*, 101-5.

Related Products

Catalog No.	Description
CLM-1396	D-Glucose (U-$^{13}C_6$, 99%)
CLM-1822-H	L-Glutamine ($^{13}C_5$, 99%)

Research Use of CIL Products

CIL manufactures highly pure research biochemicals that are produced for research applications. As a service to our customers, some of these materials have been tested for the presence of *S. aureus, P. aeruginosa, E. coli, Salmonella sp.,* aerobic bacteria, yeast and mold, as well as the presence of endotoxin in the bulk material by taking a random sample of the bulk product. Subsequent aliquots are not retested. Presence of endotoxin is assessed by determining endotoxin content following established protocols and standardized Limulus Amebocyte Lysate (LAL) reagents. These tests are provided at no charge for any materials listed in our catalog or website that is designated as "MPT" in the item product number (*e.g.,* DLM-349-MPT).

CIL is able to provide microbiological testing for other products. Depending on the compound and the quantity ordered, an additional charge may apply.

Please note that microbiological-tested products are not guaranteed to be sterile and pyrogen-free when received by the customer, and microbiological testing does not imply suitability for any desired use. If the product must be sterile and pyrogen-free for a desired application, CIL recommends that the product be packaged or formulated into its ultimate dose form by the customer or appropriate local facility. The product should always be tested by a qualified pharmacy/facility prior to actual use.

CIL research products are labeled "For Research Use Only. Not for use in diagnostic procedures." Persons intending to use CIL products in applications involving humans are responsible for complying with all applicable laws and regulations including but not limited to the US FDA, other local regulatory authorities and institutional review boards concerning their specific application or desired use.

It may be necessary to obtain approval for using these research products in humans from the US FDA or the comparable governmental agency in the country of use. CIL will provide supporting information, such as lot-specific analytical data and test method protocols, to assist medical research groups in obtaining approval for the desired use.

CIL will allocate a specific lot of a product to customers who are starting long-term projects requiring large amounts of material. Benefits from this type of arrangement include experimental consistency arising from use of only one lot, no delay in shipments, and guaranteed stock. Please note that some CIL products have a specific shelf life and cannot be held indefinitely. If interested, please contact your sales representative for further details.

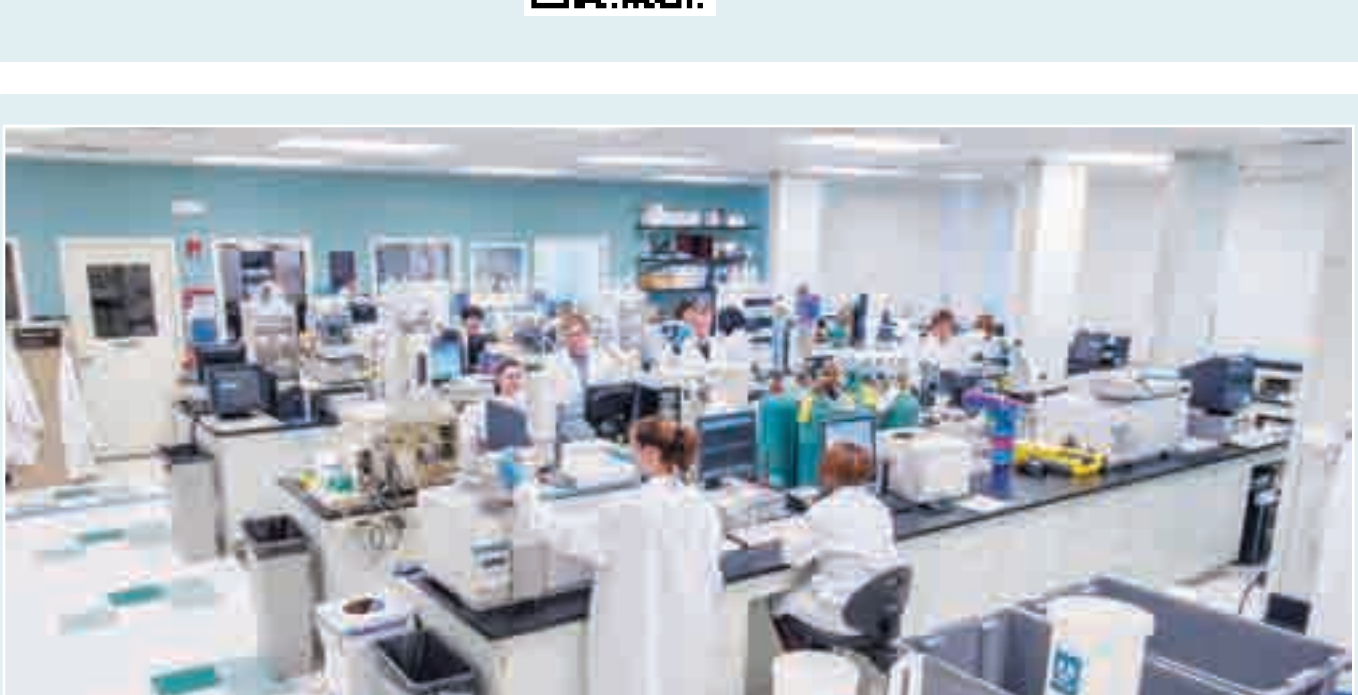
Product Quality Designation

Cambridge Isotope Laboratories, Inc. produces stable isotope-labeled products at several levels of control beyond the standard research product grade (xLM-nnn-0). These grades are designated as xLM-nnn-**MPT** and xLM-nnn-**CTM**, where "x" refers to the type of labeling (C, D, N, CN, *etc.*) and "nnn" is the catalog number. The chart on the next page shows the levels of control applied to manufacturing, quality control, quality assurance and the level of testing applied to each grade of product. The two grades of products on this chart are:

- **-MPT** – Microbiological and Pyrogen Tested. Products prepared under the -MPT classification are research-grade products that are tested in the bulk form for *S. aureus, P. aeruginosa, E. coli, Salmonella sp.,* aerobic bacteria, yeast and mold and for bacterial endotoxins.
- **-CTM** – Clinical Trial Material. Products prepared under the -CTM classification may conform to materials suitable for Phase 1 Clinical Trials as described in Section 19 of the ICH Guidance Q7A, "cGMP Guidance for Active Pharmaceutical Ingredients (APIs)." Additional data may be needed for APIs to be used in Phase 2 and Phase 3 Clinical Trials. CIL can also supply materials suitable for Phase 2 and 3 Clinical Trials.

CIL offers an Enhanced Technical Data Package (EDP) for most -MPT products. It includes all data that normally accompanies the -MPT product, plus additional information pertaining to the synthesis, purity and stability of the product. This is available for an additional charge. **Please see page 94 for further details.**

For the most up-to-date chart, please visit www.isotope.com or scan the QR code to the right.



Quality-control lab at Andover facility.

Product Quality Designation Chart

		-MPT Products	-CTM Products, Q7A Compliant
Manufacturing	Synthetic Methods	Catalog products may be prepared under SOP or following laboratory notebook procedures	Products prepared according to an approved, documented batch record
	Packaging	Performed in dedicated Packaging Dept with environmental controls. Labels are produced and reviewed by the Packaging Department. Records are maintained by the Operations and Logistics Department.	Performed in dedicated cGMP Facility with QA release. Validated and monitored environmental controls. Labels are reviewed and approved by QA with label reconciliation.
	SOPs	SOPs controlled by departmental management	Batch record and SOPs review and approved by Quality Assurance (QA)
	Change Control	Departmental management approval	Documented QA Controlled Procedure
	Raw Material Traceability	May be available upon request	Draft material specifications for all raw materials, including vendor COAs for raw materials
	Contact Glassware	Standard laboratory cleaning, glassware - multiple use	New glassware and/or glassware cleaned per cleaning verification protocol
	Facility Management	Environmentally Controlled. Certified Hoods.	Environmentally controlled cGMP Facility with room clearance procedure and/or Product Changeover Procedure
	In-Process Testing	Performed by Production or Quality Control personnel	Performed by Quality Control using scientifically sound, documented methods
	Deviations	Departmental management approval	Documented QA Controlled procedure
Quality Control	Test methods	Standard practice or written test methods	Documented, scientifically sound test methods
	SOPs	SOPs controlled by departmental management	QA Reviewed and approved
	Change Control	Departmental management approval	Documented QA Controlled procedure
	Out of Specification	Departmental management approval	Documented QA/QC Controlled procedure. Reprocessing may occur per ICH/FDA guidance and QA approval
	Deviation	Departmental management approval	Documented QA Controlled procedure
Product Quality and Release	Final Data Review	Reviewed by QC	Reviewed by QC and QA
	Certificate of Analysis	Provided by Operations and Logistics/Quality Control	Prepared/approved by QA
	Material Specifications	Determined by CIL	Material specifications agreed with customer. Approved by QA.
	USP or EP Specifications	Does not apply	Specifications and methods follow USP/EP and/or by agreement with customer
	Microbiological Testing	Bulk material tested at release for *S. aureus, P. aeruginosa, E. coli, Salmonella sp.,* aerobic bacteria, yeast and mold and for bacterial endotoxins.	Bulk material tested at release for bacterial endotoxin and USP <61> Microbial Enumeration
	BSE/TSE	Certificate may be available upon request	Certificate provided
	Retain Samples	Not required	Reserve samples of each API batch are retained for a minimum of 3 years after distribution of the batch
	Record Retention	Records are retained for a minimum of 5 years	Records are retained for a minimum of 5 years, or as defined in the customer specific agreements
	Product Stability	Not routinely tested	Not routinely tested, available by contract
	Drug Master Files	Not applicable	May be available if contracted

Notes

1. CIL -MPT products are labeled "For Research Use Only. Not for use in diagnostic procedures." CIL -CTM products are labeled "For Investigational Use Only. The performance characteristics of this product have not been established."
2. Please note that -MPT and -CTM products are not guaranteed to be sterile and pyrogen-free when received by the customer and microbiological and pyrogen testing does not imply suitability for any desired use. If the product must be sterile and pyrogen-free for a desired application, CIL recommends that the product be packaged or formulated into its ultimate dose form by the customer or appropriate local facility. The product should always be tested by a qualified pharmacy/facility prior to actual use.
3. Systems or procedures controlled by departmental management or subject to departmental management approval are the responsibility of the operating department.
4. BSE/TSE statements are developed on a risk estimate basis that meets or exceeds the guidelines laid out in section 5.2.8 European Pharmacopeia Fifth Edition. CIL does not use mammalian-sourced materials whenever possible and rarely uses materials of bovine origin.
5. Technical data packages may be available upon receipt of an executed CDA.

Microbiological and Pyrogen Tested Products

CIL offers microbiological and pyrogen testing for many of our research-grade products. For these products, denoted as -MPT, the bulk material is tested at release for *S. aureus, P. aeruginosa, E. coli, Salmonella sp.*, aerobic bacteria, yeast, mold and bacterial endotoxins. Subsequent aliquots are not retested. Microbiological testing does not imply suitability for any intended use.

For most -MPT products, CIL also offers an Enhanced Technical Data Package (EDP). It includes all data that normally accompanies the -MPT product, plus additional information pertaining to the synthesis, purity and stability of the product. This is available for an additional charge. **Please see page 94 for further details.**

Amino Acids

Catalog No.	Description
CLM-116-MPT	L-Alanine (1-^{13}C, 99%)
CLM-117-MPT	L-Alanine (3-^{13}C, 99%)
NLM-454-MPT	L-Alanine (^{15}N, 98%)
DLM-248-MPT	L-Alanine (3,3,3-D_3, 99%)
CLM-2051-MPT	L-Arginine•HCL (1,2-$^{13}C_2$, 99%)
NLM-395-MPT	L-Arginine•HCL (guanido-$^{15}N_2$, 98%+)
CLM-1801-H-MPT	L-Aspartic acid ($^{13}C_4$, 99%)
DLM-546-MPT	L-Aspartic acid (2,3,3-D_3, 98%)
CLM-4899-MPT	L-Citrulline (ureido-^{13}C, 99%)
DLM-3860-MPT	L-Citrulline (5,5-D_2, 98%)
CDLM-7879-MPT	L-Citrulline (ureido-^{13}C, 99%; 5,5-D_2, 98%)
CDLM-7139-MPT	L-Citrulline (5-^{13}C, 99%; 4,4,5,5-D_4, 95%)
CLM-3852-MPT	L-Cysteine (1-^{13}C, 99%)
DLM-769-MPT	L-Cysteine (3,3-D_2, 98%)
CLM-674-MPT	L-Glutamic acid (1-^{13}C, 99%)
CLM-1800-H-MPT	L-Glutamic acid ($^{13}C_5$, 99%)
NLM-135-MPT	L-Glutamic acid (^{15}N, 98%)
DLM-3725-MPT	L-Glutamic acid (2,4,4-D_3, 97-98%)
CLM-3612-MPT	L-Glutamine (1-^{13}C, 99%)
CLM-2001-MPT	L-Glutamine (1,2-$^{13}C_2$, 99%)
CLM-1822-H-MPT	L-Glutamine ($^{13}C_5$, 99%)
NLM-557-MPT	L-Glutamine (amide-^{15}N, 98%+)
NLM-1016-MPT	L-Glutamine (α-^{15}N, 98%)
NLM-1328-MPT	L-Glutamine ($^{15}N_2$, 98%)
CLM-422-MPT	Glycine (1-^{13}C, 99%)
CLM-136-MPT	Glycine (2-^{13}C, 99%)
CLM-1017-MPT	Glycine (1,2-$^{13}C_2$, 97-99%)
NLM-202-MPT	Glycine (^{15}N, 98%)
DLM-1674-MPT	Glycine (2,2-D_2, 98%)
CNLM-1673-H-MPT	Glycine ($^{13}C_2$, 99%; ^{15}N, 99%)
CLM-1026-MPT	L-Isoleucine (1-^{13}C, 99%)
CLM-468-MPT	L-Leucine (1-^{13}C, 99%)
CLM-3524-MPT	L-Leucine (1,2-$^{13}C_2$, 99%)
CLM-2262-H-MPT	L-Leucine ($^{13}C_6$, 99%)
NLM-142-MPT	L-Leucine (^{15}N, 98%)
DLM-1259-MPT	L-Leucine (5,5,5-D_3, 99%)
DLM-4212-MPT	L-Leucine (isopropyl-D_7, 98%)
DLM-567-MPT	L-Leucine (D_{10}, 98%)
CNLM-615-MPT	L-Leucine (1-^{13}C, 99%; ^{15}N, 98%+)
CNLM-281-H-MPT	L-Leucine ($^{13}C_6$, 99%; ^{15}N, 99%)
ULM-8203-MPT	L-Leucine (unlabeled)

Catalog No.	Description
CLM-653-MPT	L-Lysine•2HCl (1-^{13}C, 99%)
NLM-143-MPT	L-Lysine•2HCl (α-^{15}N, 95-99%)
DLM-2640-MPT	L-Lysine•2HCl (4,4,5,5-D_4, 96-98%)
CLM-206-MPT	L-Methionine (methyl-^{13}C, 99%)
CLM-3267-MPT	L-Methionine (1-^{13}C, 99%)
CLM-893-H-MPT	L-Methionine ($^{13}C_5$, 99%)
DLM-431-MPT	L-Methionine (methyl-D_3, 98%)
CDLM-760-MPT	L-Methionine (1-^{13}C, 99%; methyl-D_3, 98%)
CLM-1036-MPT	L-Ornithine•HCl (1,2-$^{13}C_2$, 99%)
CLM-762-MPT	L-Phenylalanine (1-^{13}C, 99%)
CLM-1055-MPT	L-Phenylalanine (ring-$^{13}C_6$, 99%)
CLM-2250-H-MPT	L-Phenylalanine ($^{13}C_9$, 99%)
NLM-108-MPT	L-Phenylalanine (^{15}N, 98%)
DLM-1258-MPT	L-Phenylalanine (ring-D_5, 98%)
DLM-372-MPT	L-Phenylalanine (D_8, 98%)
ULM-8205-MPT	L-Phenylalanine (unlabeled)
CLM-510-MPT	L-Proline (1-^{13}C, 99%)
NLM-835-MPT	L-Proline (^{15}N, 98%)
CNLM-436-H-MPT	L-Proline ($^{13}C_5$, 99%; ^{15}N, 99%)
ULM-8333-MPT	L-Proline (unlabeled)
CLM-1573-MPT	L-Serine (1-^{13}C, 99%)
CLM-1572-MPT	L-Serine (3-^{13}C, 99%)
CLM-2261-MPT	L-Threonine ($^{13}C_4$, 97-99%)
NLM-742-MPT	L-Threonine (^{15}N, 98%)
CNLM-587-MPT	L-Threonine ($^{13}C_4$, 97-99%; ^{15}N, 97-99%)
CLM-778-MPT	L-Tryptophan (1-^{13}C, 99%)
DLM-1092-MPT	L-Tryptophan (indole-D_5, 98%)
CLM-776-MPT	L-Tyrosine (1-^{13}C, 99%)
CLM-1542-MPT	L-Tyrosine (ring-$^{13}C_6$, 99%)
NLM-590-MPT	L-Tyrosine (^{15}N, 98%)
DLM-2317-MPT	L-Tyrosine (3,3-D_2, 98%)
DLM-449-MPT	L-Tyrosine (ring-3,5-D_2, 98%)
DLM-451-MPT	L-Tyrosine (ring-D_4, 98%)
CLM-470-MPT	L-Valine (1-^{13}C, 99%)
CLM-2249-H-MPT	L-Valine ($^{13}C_5$, 99%)
NLM-316-MPT	L-Valine (^{15}N, 98%)
DLM-488-MPT	L-Valine (D_8, 98%)

MPT = *microbiological/pyrogen tested*

Carbohydrates

Catalog No.	Description
CLM-1201-MPT	D-Fructose (1-^{13}C, 99%)
CLM-1553-MPT	D-Fructose (U-$^{13}C_6$, 99%)
CLM-744-MPT	D-Galactose (1-^{13}C, 99%)
DLM-1390-MPT	D-Galactose (1-D, 98%)
CLM-420-MPT	D-Glucose (1-^{13}C, 98-99%)
CLM-746-MPT	D-Glucose (2-^{13}C, 99%)
CLM-1393-MPT	D-Glucose (3-^{13}C, 99%)
CLM-504-MPT	D-Glucose (1,2-$^{13}C_2$, 99%)
CLM-2717-MPT	D-Glucose (1-^{13}C, 99%; 6-^{13}C, 97%+)
CLM-6750-MPT	D-Glucose (3,4-$^{13}C_2$, 99%)
CLM-1396-MPT	D-Glucose (U-$^{13}C_6$, 99%)
DLM-1150-MPT	D-Glucose (1-D, 98%)
DLM-1271-MPT	D-Glucose (2-D, 98%)
DLM-349-MPT	D-Glucose (6,6-D_2, 99%)
DLM-2062-MPT	D-Glucose (1,2,3,4,5,6,6-D_7, 98%)
CDLM-3813-MPT	D-Glucose (U-$^{13}C_6$, 99%; 1,2,3,4,5,6,6-D_7, 97-98%)
CLM-1189-MPT	D-Mannitol (1-^{13}C, 98%)
CLM-8091-MPT	D-Sucrose (glucose-$^{13}C_6$, 98%)
CLM-2456-MPT	D-Xylose (1,2-$^{13}C_2$, 99%)

Fatty Acids and Lipids

Catalog No.	Description
CLM-3960-MPT	Linoleic acid, ethyl ester (linoleate-U-$^{13}C_{18}$, 98%+) CP 95%
CLM-6229-MPT	Linoleic acid, potassium salt (1-^{13}C, 99%)
DLM-2351-MPT	Linolenic acid, ethyl ester (17,17,18,18,18-D_5, 98%) **microbiological tested only**, CP 90%
DLM-2351-P-MPT	Linolenic acid, potassium salt (17,17,18,18,18-D_5, 98%) CP 95%
CLM-8455-MPT	Mixed fatty acids (^{13}C, 98%+)
CLM-293-MPT	Octanoic acid (1-^{13}C, 99%)
DLM-619-MPT	Octanoic acid (D_{15}, 98%)
CLM-149-MPT	Oleic acid (1-^{13}C, 99%)
DLM-689-MPT	Oleic acid (9,10-D_2, 97%)
CLM-4477-MPT	Oleic acid, potassium salt (1-^{13}C, 99%)
CLM-6230-MPT	Oleic acid, sodium salt (1-^{13}C, 99%)
CLM-150-MPT	Palmitic acid (1-^{13}C, 99%)
CLM-409-MPT	Palmitic acid (U-$^{13}C_{16}$, 98%)
DLM-2893-MPT	Palmitic acid (7,7,8,8-D_4, 98%)
DLM-215-MPT	Palmitic acid (D_{31}, 98%)
CLM-1889-MPT	Potassium palmitate (1-^{13}C, 99%)
CLM-3943-MPT	Potassium palmitate (U-$^{13}C_{16}$, 98%+)
DLM-3773-MPT	Potassium palmitate (2,2-D_2, 97%)
DLM-6033-MPT	Potassium palmitate (7,7,8,8-D_4, 98%)
CLM-1948-MPT	Sodium octanoate (1-^{13}C, 99%)
CLM-174-MPT	Sodium palmitate (1-^{13}C, 99%)
CLM-6059-MPT	Sodium palmitate ($^{13}C_{16}$, 98%+)
CLM-163-MPT	Triolein (1,1,1-$^{13}C_3$, 99%)
CLM-164-MPT	Tripalmitin (1,1,1-$^{13}C_3$, 99%) **microbiological tested only**
CLM-8445-MPT	Tripalmitin (glyceryl-$^{13}C_3$, 99%) **microbiological tested only**

MPT = *microbiological/pyrogen tested*

Other Tracers

Catalog No.	Description
CLM-630-MPT	Aminopyrine (*N,N*-dimethyl-$^{13}C_2$, 99%)
CLM-1813-MPT	Benzoic acid (ring-$^{13}C_6$, 99%)
CLM-728-MPT	Caffeine (3-methyl-^{13}C, 99%)
CLM-1608-MPT	Chloral hydrate (trichloromethyl-^{13}C, 97%)
CLM-804-MPT	Cholesterol (3,4-$^{13}C_2$, 99%)
DLM-3057-MPT	Cholesterol (25,26,26,26,27,27,27-D_7, 98%)
DLM-549-MPT	Choline chloride (trimethyl-D_9, 97-98%)
CLM-7933-MPT	Creatine (guanidino-^{13}C, 99%)
DLM-1302-MPT	Creatine (methyl-D_3, 98%)
CLM-7401-MPT	L-Dopa (1-^{13}C, 99%)
CLM-7824-MPT	L-Dopa (1-^{13}C, ring-$^{13}C_6$, 99%)
DLM-2259	Deuterium oxide (D, 99.8%) **sterility tested**
DLM-2259-70	Deuterium oxide (D, 70%) **sterility tested**
CLM-3758-MPT	Erythromycin, lactobioante salt (*N,N*-dimethyl-$^{13}C_2$, ~90%)
CLM-344-MPT	Ethanol (1-^{13}C, 99%) (<6% H_2O)
CLM-3297-MPT	Ethyl acetoacetate (1,2,3,4-$^{13}C_4$, 99%)
CLM-1397-MPT	Glycerol (2-^{13}C, 99%)
CLM-1510-MPT	Glycerol ($^{13}C_3$, 99%)
DLM-1229-MPT	Glycerol (1,1,2,3,3-D_5, 99%)
CLM-191-MPT	Glycocholic acid (glycine-1-^{13}C, 99%)
CLM-8730-MPT	2-Hydroxybenzoic acid (salicylic acid) (ring-$^{13}C_6$, 99%)
CLM-2093-MPT	α-Ketoisocaproic acid, sodium salt (1-^{13}C, 99%)
DLM-1944-MPT	α-Ketoisocaproic acid, sodium salt (methyl-D_3, 98%)
CNLM-7633-MPT	Lamotrigine (5,6-$^{13}C_2$, 99%; 5-amino-^{15}N, 98%)
CLM-7522-MPT	Naproxen, sodium salt (*O*-methyl-^{13}C, 98%)
NLM-1048-MPT	Orotic acid•H_2O (1,3-$^{15}N_2$, 98%+)
CLM-4449-MPT	Oxalic acid, disodium salt (1,2-$^{13}C_2$, 99%)
CLM-156-MPT	Sodium acetate (1-^{13}C, 99%)
CLM-381-MPT	Sodium acetate (2-^{13}C, 99%)
CLM-440-MPT	Sodium acetate (1,2-$^{13}C_2$, 99%)
DLM-3126-MPT	Sodium acetate (D_3, 99%)
CLM-441-MPT	Sodium bicarbonate (^{13}C, 99%), CP 97%
CLM-1256-MPT	Sodium butyrate (1-^{13}C, 99%)
DLM-7616-MPT	Sodium butyrate (D_7, 98%)
CLM-3780-MPT	Sodium dichloroacetate ($^{13}C_2$, 99%)
CLM-3706-MPT	Sodium D-3-hydroxybutyrate (2,4-$^{13}C_2$, 99%)
CLM-1577-MPT	Sodium L-lactate (1-^{13}C, 99%) (20% w/w in H_2O)
CLM-1578-MPT	Sodium L-lactate (3-^{13}C, 98%) (20% w/w in H_2O)
CLM-1579-MPT	Sodium L-lactate ($^{13}C_3$, 98%) (20% w/w in H_2O)
DLM-4353-MPT	Sodium L-lactate (2-D, 98%) (20% w/w in H_2O)
CLM-771-MPT	Sodium propionate (1-^{13}C, 99%)
CLM-1865-MPT	Sodium propionate ($^{13}C_3$, 99%)
DLM-1601-MPT	Sodium propionate (D_5, 98%)
CLM-1082-MPT	Sodium pyruvate (1-^{13}C, 99%)
CLM-1575-MPT	Sodium pyruvate (3-^{13}C, 99%)
DLM-2949-MPT	Tau-methyl-L-histidine (methyl-D_3, 98%)
NLM-3901-MPT	Thymidine ($^{15}N_2$, 96-98%), CP 97%
CLM-7491-MPT	*cis*-(+/-)-Tramadol•HCl (methoxy-^{13}C, 99%)
CLM-3276-MPT	Uracil (2-^{13}C, 99%)
CLM-311-MPT	Urea (^{13}C, 99%)
NLM-233-MPT	Urea ($^{15}N_2$, 98%+)
COLM-4861-MPT	Urea (^{13}C, 99%; ^{18}O, 98%)
NLM-1697-MPT	Uric acid (1,3-$^{15}N_2$, 98%+)

Enhanced Technical Data Package (EDP)

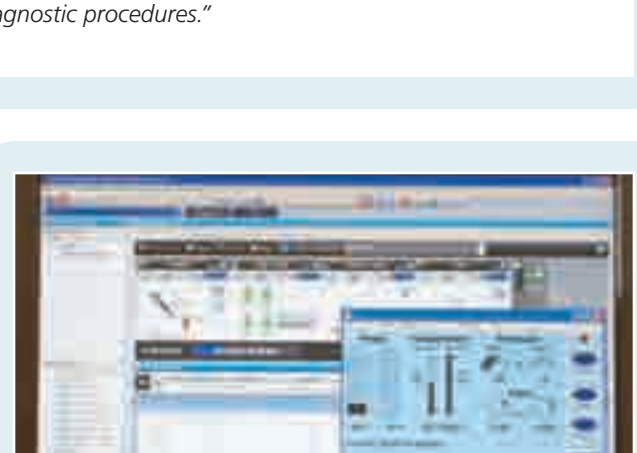
To better serve our customers' needs, CIL provides the option of an Enhanced Technical Data Package. This data package is available for most -MPT products. It includes all of the data currently included with the -MPT products, as well as the additional information listed below.

Our customers have the option of purchasing this package at the time of order or at a later date. *Please note that if you purchase at a later date, some of the information listed below may not be available. Also, the EDP may not be available for all lots; availability to be confirmed prior to order.*

Enhanced Technical Data Package Contents

1. Additional testing data: products with an EDP have been tested to the specifications detailed in USP or EP, but not using compendia methods.
2. Product description: structural formula, stereochemical description, molecular formula.
3. Product physical properties: melting point, pH, optical rotation (mix of literature and measured values).
4. Outline of the synthesis route, including details of solvents used.
5. Data used to confirm structure and chemical purity.
6. Impurity profile: available data on impurities detected and identified together with the method of detection and the cutoff applied.
7. Residual solvents: measured residual solvents from the final synthetic step and purification.
8. Certificates of Analysis: raw materials as appropriate.
9. Stability data: estimated and measured.
    a. This will be either actual shelf life data, if it can be obtained from CIL history or by analysis of in-stock batches or,
    b. If no data is available, CIL will commit to assaying the batch provided after six months and one year. Data will be provided after one year, unless the batch fails assay after six months. This option will not be available if the Enhanced Data Package is ordered at a later date.

CIL products are labeled "For Research Use Only – not for use in diagnostic procedures."



cGMP Capabilities

Cambridge Isotope Laboratories, Inc. has been routinely manufacturing cGMP products since 1994 and has been continuously increasing the cGMP product offering throughout the years in an effort to support clinical research. CIL is ISO 13485 certified and its facilities are inspected by the FDA on an ongoing basis. CIL's state-of-the-art cGMP production and quality control suite occupies over 10,000 square feet in CIL's Tewksbury, MA, location.

All customers receive a wide range of support throughout each cGMP project. A CIL project coordinator serves as a liaison to ensure accurate and timely communication between the customer and CIL. An additional support team of experts specializing in synthetic chemistry, quality control and quality assurance serves to provide technical guidance for the customer from beginning to end.

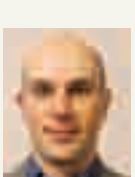
Each cGMP product is manufactured, tested, packaged and released according to current Good Manufacturing Practice. The products are tested by the Quality Control Department and meet or exceed specifications as outlined by the USP and/or EP, or as determined by the customer. Stability studies on cGMP products are routinely performed following ICH guidelines. If a Drug Master File (DMF) or other documentation to support regulatory filing is required by the customer, it can be prepared and maintained by CIL's Quality Assurance Department. Additional fees may apply for stability studies or regulatory information.

Products prepared under the –CTM classification conform to materials suitable for Phase I Clinical Trials as described in Section 19 of the ICH Guidance Q7A, "cGMP Guidance for Active Pharmaceutical Ingredients (APIs)." Q7A is recognized by the European, Japanese and US authorities. CIL is also capable of producing materials suitable for Phase 2 and Phase 3 Clinical Trials.

Partial Listing of cGMP Products

- Water (^{18}O, 97%)
- Urea (^{13}C, 99%)
- Uracil (2-^{13}C, 98%)
- Cholesterol (3,4-$^{13}C_2$, 99%)
- Deuterium oxide (D, 70%)
- Dextromethorphan•HBr•H_2O (*O*-methyl-^{13}C, 98%)
- D-Glucose (6,6-D_2, 99%)
- D-Glucose ($^{13}C_6$, 99%)
- Glycerol (1,1,2,3,3-D_5, 99%)
- L-Leucine ($^{13}C_6$, 99%)
- L-Leucine (5,5,5-D_3, 98%)
- (+/-)-Pantoprazole, sodium salt: sesquihydrate (pyridyl-4-methoxy-^{13}C, 98%)
- Sodium acetate (1-^{13}C, 99%)
- Sodium acetate (1,2-$^{13}C_2$, 98%)
- Phenylalanine (1-^{13}C, 99%)

Other compounds can be manufactured under cGMP conditions. Please inquire about the compound of interest.

Stable Isotope Labeling Kinetics (SILK™) to Measure the Metabolism of Brain-Derived Proteins Implicated in Neurodegeneration

Joel B. Braunstein, MD, MBA and Tim West, PhD
C_2N Diagnostics, LLC; Center for Emerging Technologies, St. Louis, MO 63108 USA

Alzheimer's disease (AD) is a progressive neurodegenerative disease on track to becoming one of the greatest challenges to the healthcare system in the 21st century. AD affects millions of people in one way or another. It causes long-term memory loss, confusion, mood swings, and, eventually, loss of bodily functions. Sufferers from Alzheimer's tend to withdraw from family, friends and other members of society as symptoms worsen. To date, there are no known cures, and patients and families of patients struggle with symptoms until death. Recent research has shown hope for early diagnosis and treatment. Much of this research has focused on amyloid plaques that are present in the brains of Alzheimer's patients. One approach to studying this unnatural accumulation of amyloid plaques is to monitor synthesis and clearance of the beta-amyloid peptide (Aβ) using L-Leucine ($^{13}C_6$, 99%) (CLM-2262-H).

Quantifying alterations in protein synthesis and clearance rates is vital to understanding disease pathogenesis. It also enables a determination of the effects of novel drug treatments on target protein metabolism. The powerful combination of *in vivo* stable isotope labeling and mass spectrometry has made this possible. Specifically, researchers at Washington University have developed a proprietary method to measure the metabolism of Aβ and other proteins in the human central nervous system (CNS). C_2N Diagnostics, LLC, has commercialized this platform for use in CNS drug development, disease detection and progression monitoring.

Alzheimer's neurons with amyloid plaque.

In this method, individuals receive an administration of L-Leucine ($^{13}C_6$, 99%) followed by serial cerebrospinal fluid (CSF) and plasma sampling. The clinical site that obtains these biological samples then sends them off to a central laboratory (*i.e.,* at C_2N Diagnostics) for processing and analysis. Mass spectrometry quantifies the [$^{13}C_6$] leucine enrichment of Aβ to obtain rates of amyloid production and degradation. The SILK™ platform can also assess the kinetics of apolipoproteinE (apoE) in cell culture as well as the human brain, among other proteins implicated in neurodegeneration. ApoE is the greatest known genetic risk factor for late-onset Alzheimer's disease. Elucidating the metabolism of the various apoE isoforms is beginning to provide important insights about the role that apoE plays in the disease progression of AD.

The SILK™ platform enables the testing of Alzheimer's drugs *in vivo* to determine the effects of the drug on the CNS and other systems in the body. This information is beneficial as a therapeutic biomarker for use in early clinical development. It has the potential to halt undeserving drug candidates early during the development process; thereby, reducing high downstream costs and wasted time to pharmaceutical companies.

Since most leucine-containing proteins are labeled after [$^{13}C_6$] leucine infusion, this robust and versatile technique can be used as a method to determine the turnover rates for many different proteins. It can identify and quantify potential biomarkers for diseases and metabolic disorders beyond Alzheimer's. Please see the list at right for peer-reviewed references that describe the utility of this method.

Selected Publications

Huang, Y.; Potter, R.; Sigurdson, W.; Kasten, T.; Connors, R.; Morris, J.C.; Benzinger, T.; Mintun, M.; Ashwood, T.; Ferm, M.; Budd, S.L.; Bateman, R.J. **2012**. β-Amyloid Dynamics in Human Plasma. *Arch Neurol, 69,* 1591-7.

Wildsmith, K.R.; Basak, J.M.; Patterson, B.W.; Pyatkivskyy, Y.; Kim., J.; Yarasheski, K.E.; Wang, J.X.; Mawuenyega, K.G.; Jiang, H.; Parsadanian, M.; Yoon, H.; Kasten, T.; Sigurdson, W.C.; Xiong, C.; Goate, A.; Holtzman, D.M.; Bateman, R.J. **2012**. *In vivo* human apolipoprotein E isoform fractional turnover rates in the CNS. *PLoS One, 7*, e38013. Epub 2012.

Castellano, J.M.; Kim, J.; Stewart, F.R.; Jiang, H.; Demattos, R.B.; Patterson, B.W.; Fagan, A.M.; Morris, J.C.; Mawuenyega, K.G.; Cruchaga, C.; Goate, A.M.; Bales, K.R.; Paul, S.M.; Bateman, R.J.; Holtzman, D.M. **2011**. Human apoE Isoforms Differentially Regulate Brain Amyloid-Peptide Clearance. *Sci Transl Med, 3,* 89ra57. PMC Journal – In Process. PMID#21715678.

Huang, Y.; Potter, R.; Sigurdson, W.; Santacruz, A.; Shih, S.; Ju, Y.; Kasten, T.; Morris, J.C.; Mintun, M.; Duntley, S.; Bateman, R.J. **2011**. Effects of Age and Amyloid Deposition on Aβ Dynamics in the Human Central Nervous System. *Archives of Neurology*. Published online September 12, 2011. doi:10.1001/archneurol.2011.235. NIHMS326112.

Bateman, R.J.; Aisen, P.S.; De Strooper, B.; Fox, N.C.; Lemere, C.A.; Ringman, J.M.; Salloway, S.; Sperling, R.A.; Windisch, M.; Xiong, C. **2011**. Autosomal-dominant Alzheimer's disease: a review and proposal for the prevention of Alzheimer's disease. *Alzheimer's Res Ther, 2,* 35. PMCID: PMC3109410.

Mawuenyega, K.G.; Sigurdson, W.; Ovod, V.; Munsell, L.; Kasten, T.; Morris, J.C.; Yarasheski, K.E.; Bateman, R.J. **2010**. Decreased Clearance of CNS Amyloid-β in Alzheimer's disease. *Science, 330,* 1774.

Wildsmith, K.R.; Han, B.; Bateman, R.J. **2009**. Method for the simultaneous quantitation of apolipoprotein E isoforms using tandem mass spectrometry. *Anal Biochem, 395,* 116-8.

Elbert, D.L.; Mawuenyega, K.G.; Scott, E.A.; Wildsmith, K.R.; Bateman, R.J. **2008**. Stable Isotope Labeling Tandem Mass Spectrometry (SILT): Integration with Peptide Identification and Extension to Data-Dependent Scans. *J Proteome Res, 7,* 4546-56.

Bateman, R.J.; Munsell, L.Y.; Chen, X.; Holtzman, D.M.; Yarasheski, K.E. **2007**. Stable Isotope Labeling Tandem Mass Spectrometry (SILT) to Quantify Protein Production and Clearance Rates. *J Am Soc Mass Spectrom, 18,* 997-1006.

Bateman, R.J.; Wen, G.; Morris, J.C.; Holtzman, D.M. **2007**. Fluctuations of CSF amyloid-beta levels: Implications for a diagnostic and therapeutic biomarker. *Neurology, 68,* 666-9.

Related Product

Catalog No.	Description
CLM-2262-H	L-Leucine ($^{13}C_6$, 99%)

Researcher Perspective

Stable Isotopes in Drug Development and Personalized Medicine: Biomarkers that Reveal Causal Pathway Fluxes and the Dynamics of Biochemical Networks

Marc Hellerstein, MD, PhD
Professor of Human Nutrition (Calloway Chair), University of California at Berkeley
Professor of Endocrinology, Metabolism and Nutrition, University of California at San Francisco
Co-Founder and Chief of SAB, KineMed, Inc., Emeryville, CA 94608 USA

The combination of stable isotope labeling with powerful mass spectrometric analytic techniques is providing increasingly important diagnostic tools for drug development and clinical diagnostics in the emerging era of personalized medicine.

The Problem: High Attrition Rates in Contemporary Drug Development

Although it is widely believed that we live in a golden era of breakthroughs in new medicines, the opposite is true. Recent years have witnessed the lowest rate of new drug approvals in a generation, despite greatly increased pharmaceutical industry investment.[1] These disappointing facts hold true for all classes of disease, but are particularly worrisome for growing epidemics of chronic disease, such as Alzheimer's disease, diabetes, osteoarthritis and obesity-related disorders.

The problem is not a lack of molecular targets or candidate drugs. The molecular target-based approach to drug discovery, which has dominated pharmaceutical research for the past 20 years, has generated huge lists of genes, proteins and potential drug therapies.

The problem is that the attrition rate of drug leads has gotten worse, not better, with >98% of leads now failing for efficacy or safety reasons, including 90% failure rates in human trials.[2,3] This attrition is largely responsible for the high cost of each successful drug eventually approved.

Losing the War with Complexity

Attrition, in turn, is largely due to the unpredictability of the complex networks that comprise living systems in response to targeted interventions at specific nodes.[2] Unanticipated functional consequences of targeted interventions, both undesirable and beneficial, are the rule rather than the exception in such systems (Figure 1). Pathogenic heterogeneity among individuals within each disease magnifies this problem, requiring different intervention strategies for different subsets of patients. The latter issue is embodied by the notion of personalized medicine.

The Missing Link: Metrics for Navigating through the Complex Biology of Disease

The key missing factors for navigating through the complex biology of disease are objective measures that guide drug developers toward the goals of safe and efficacious outcomes.[4] These metrics, called biomarkers, must be predictive of clinical outcomes and translatable from preclinical models into humans. The most reliable way to achieve these goals is to capture the underlying biologic processes driving each disease (*i.e.* the disease modifying pathways or underlying pathogenesis). Metrics of this type can serve to guide rational drug discovery and development and allow monitoring of clinical response.

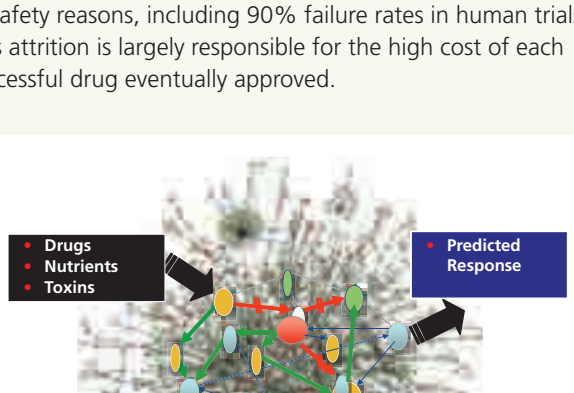


Figure 1. Losing the War with Complexity: Unpredictability of Complex Dynamic Networks.

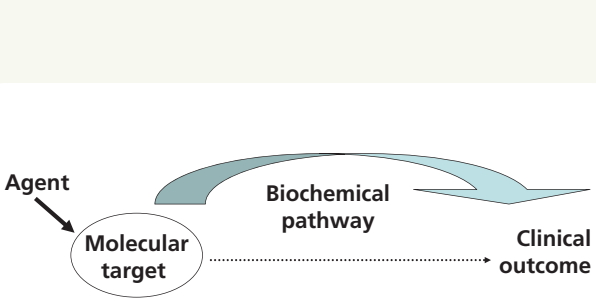


Figure 2. Pathway Fluxes as the Link Between Molecular Targets and Clinical Outcomes.

Nowhere will this need for functionally informative biomarkers be greater than in the field of "personalized medicine"–the right patient, the right drug, at the right time, and in the right dose. Companion diagnostic tests are extremely high value examples of this trend.

Stable Isotopes Are Essential for a New Class of Biomarkers: Tests that Predict Clinical Outcomes by Revealing Functionally Interpretable Information about Underlying Disease Processes

A new class of biomarkers is needed that are predictive of clinical outcomes.[4,5] The biologic pathways that underlie chronic diseases – the causal processes responsible for initiation, progression, severity and therapeutic reversal of disease – generally involve the flow of molecules through a pathway that is itself complex and influenced by numerous factors[5-8] (Figure 2).

Stable isotopic techniques have made all of these causal pathways measurable in higher organisms.

What Stable Isotopes Bring to Diagnostic Biomarkers

In the following discussion, the underlying principles and recent examples of stable isotope-based biomarkers will be briefly reviewed.

Stable isotopes allow fluxes through metabolic pathways and the dynamics of global biochemical networks to be measured, without toxicity and often non-invasively, for two reasons: first, experimental administration of stable isotopes introduces an "asymmetry" in the dimension of time (label not present, then present), which allows the timing of dynamic processes to be measured; and, second, biochemical research over the past century has established the pathways that link molecules in cells and organisms, allowing the fates of labeled substrates to be traced *in vivo*.

Importantly, stable isotopes have been used for over 70 years in humans and experimental animals and have almost no known toxicities. The FDA policy toward stable isotope-labeled products is clear and has been consistent for decades: no regulatory approval is required to administer stable isotope-labeled compounds, beyond what is needed to administer their natural abundance congeners (sterility, pyrogenicity, *etc.*). It should be noted that stable isotopic-mass spectrometric biomarkers are not radiographic imaging techniques, but require a sample from the body (blood, urine, CSF, tissue, saliva).

Two Broad Categories of Stable Isotope-Based Kinetic Biomarkers Are Available

There are two broad categories of stable isotope-based biomarkers that are most useful in drug development and diagnostics: (1) Kinetics of targeted causal pathways and, (2) Interrogation of network dynamics for unbiased discovery of kinetic signatures and unanticipated causal pathways. Both types are available and useful in drug discovery and development.[5-16]

Table 1. Examples of Causal Pathways:

A) Neurobiology

- Cargo transport through axons
- Amyloid beta synthesis and plaque turnover
- Neurogenesis
- Myelination/remyelination
- Neurotransmitter release and turnover
- Neuronal mitochondrial biogenesis
- Neuroinflammation, microglia activation
- Cytokine release
- Hungtingtin protein turnover
- Prion turnover
- Synaptic plasticity

B) Obesity/T2DM

- Pancreatic beta cell proliferation and mass
- Insulin-mediated glucose uptake
- Hepatic glucose production
- Adipogenesis and TG deposition
- Adipose tissue fatty acid oxidation/brown fat transition
- Adipose tissue remodeling
- Hepatic TG synthesis and release
- Atheroma cholesterol removal and deposition
- Adipose tissue macrophage proliferation and activation
- Muscle mitochondrial beta-oxidation and biogenesis

C) Cancer/Neoplasia

- Tumor cell proliferation and death rate
- Angiogenesis
- Lymphangiogenesis/metastatic spread
- Tumor-specific T-cell proliferation
- DNA methylation/demethylation
- Ribonucleotide reductase activity
- Histone deacetylation
- Precancer evolution to aggressive phenotypic
- Extracellular matrix turnover

(continued)

Kinetics of Targeted Causal Pathways as Biomarkers for Drug Discovery and Development

Some common examples of causal pathways in disease are shown (Table 1). These include: synthesis of collagen and extracellular matrix in fibrotic diseases; myelin synthesis and metabolism in multiple sclerosis; turnover of amyloid plaque and synthesis of amyloid beta 1-42 in Alzheimer's disease; synthesis of muscle myosin and biogenesis of mitochondria in sarcopenia; angiogenesis and proliferation and death of tumor cells in cancer; transport of cargo molecules through axons in neurodegenerative conditions; autophagic flux in Huntington's, Parkinson's and other diseases characterized by protein aggregates; clot formation and lysis in thromboembolic diseases; insulin-mediated glucose uptake and pancreatic beta cell proliferation in insulin-resistant states; adipose tissue lipid dynamics and remodeling in obesity; reverse cholesterol transport in atherosclerosis; activation of the complement cascade in inflammatory states; HIV replication and turnover of CD4+T-cells in AIDS; and many others.

The ability to measure the activity of any of these functionally relevant processes that are believed to play causal roles in disease is potentially transformative for drug discovery and development in these fields (*e.g.* Parkinson's Disease.[10,11]).

Interrogation of Network Dynamics

Perhaps the most exciting advance in stable isotope biomarkers in recent years is the emergence of "Network Dynamics": unbiased interrogation of the dynamic behavior of complex biochemical networks that comprise living systems. This has been successfully applied to preclinical models and humans for the dynamics of the global proteome, or Dynamic Proteomics.[12,13] This provides a new type of systems biology, with great potential as an unbiased screening tool for biomarker discovery.

Dynamic Proteomics represents the most functionally interpretable of the "omics" technologies – *i.e.*, providing not just heat maps or informatics, but functionally interpretable systems biology information. The operational flow chart for measuring the dynamics of a proteome is shown (Figure 3). This approach has been applied with great success to questions such as the effects of calorie restriction of cellular proteostasis, including mitochondrial biogenesis and mitophagy; the proteome dynamic signature of poor prognosis in chronic lymphocytic leukemia tumor cells; differentiating between pancreatic islets successfully compensating for insulin resistance in obese animals vs. islets that are failing and becoming "exhausted"; the effects of exercise on muscle proteome turnover; the effects of neuro-inflammation on CSF proteome turnover; the dynamics of the high-density lipoproteins (HDL) proteome in dyslipidemic states; and other questions of interest in physiology and pathophysiology.

'Virtual Biopsy' Approach for Non-Invasive Biomarkers of Intracellular Pathways

Unbiased screening of proteome dynamics in a tissue can also lead to discovery of targeted protein biomarkers that are accessible to sampling in a body fluid. Called the "virtual biopsy" technique (Figure 4), this is a powerful method for measuring the rate of protein synthesis or protein breakdown in an inaccessible tissue of origin, such as skeletal muscle, heart, brain, kidney, liver, or a cancer tissue, through a measurement made from an accessible body fluid, such as blood, cerebrospinal fluid, saliva or urine. The method comprises administering a stable isotope tracer (*e.g.* Deuterium oxide (D, 70%) (DLM-4-70); L-Leucine ($^{13}C_6$, 99%) (CLM-2262); Glycine (^{15}N, 98%) (NLM-202); Spirulina whole cells (lyophilized powder) (U-^{15}N, 98%+) (NLM-8401)) that is metabolically incorporated into newly synthesized proteins. These proteins then escape into an accessible body fluid, from which they are isolated and analyzed for isotopic content or pattern. The measured replacement rate of the escaped protein reflects the synthesis or breakdown rate of the protein back in the tissue of origin. A "virtual biopsy" of the tissue of origin has thereby been carried out.

The "virtual biopsy" method has utility for discovering and validating biomarkers for use in drug discovery and development, for identifying disease subsets in personalized medicine and for clinical diagnosis and management of patients. This approach has been developed and applied to blood-based measurements of tissue fibrosis and skeletal muscle protein synthesis and CSF-based measurements of axonal transport of cargo[10] and neuro-inflammation. An example is plasma creatine kinase-MM (derived from skeletal muscle), for measuring skeletal muscle protein anabolism from a blood test. Many other applications can be envisioned.

In Situ *Kinetic Histochemistry: Combining Histopathology with Stable Isotopes and Mass Spectrometry*

It is also now possible to visualize the kinetics of targeted molecules of interest spatially, within a histopathologic specimen.[14] Linking spatial histologic information with molecular flux rates provides a remarkable new dimension to pathologic diagnosis and monitoring of disease. This can be carried out by either laser microdissection or physical microdissection of slides (Figure 5). An example of tissue microdissection after introducing stable isotopes has been published for prostate cancer. The proliferative gradient of prostate cells, for example, has been shown to correlate closely with histologic grade in biopsy specimens from men with prostate cancer and is reflected by the proliferative rate of prostate epithelial cells isolated from seminal fluid, as a potential non-invasive biomarker.[14]

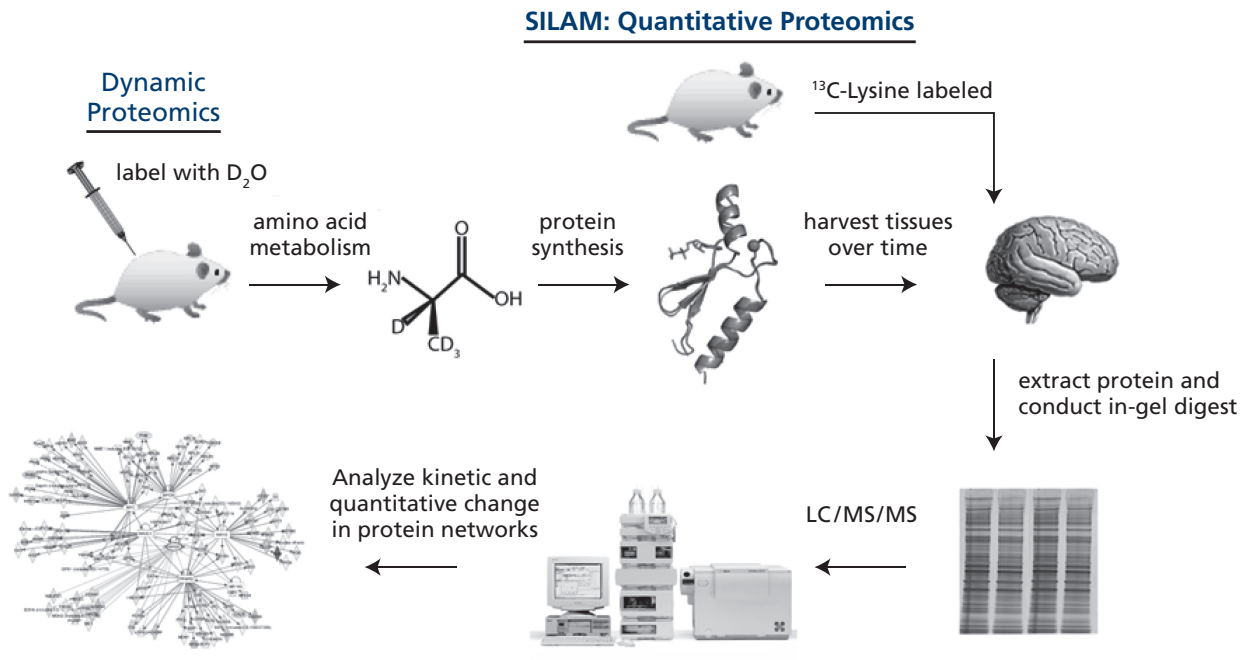


Figure 3. Dynamic Proteomics: Measuring Proteome Kinetics and Concentrations via Stable Isotope Labeling *in Vivo*.

Kinetic Imaging of Tissue Samples

Kinetic or metabolic flux imaging is now possible by combining stable isotope labeling with mass spectrometric imaging of tissues, through NIMS or MALDI-based spatial visualization of histologic slides. Spatially defined kinetic lipidomics in cancer models has revealed anatomic differences in tumor behavior that correlate with *in vivo* aggressiveness in mouse mammary cancer models.[15]

Practical Uses of Stable Isotope-Based Biomarkers in Drug Development

There are many uses for stable isotope-based biomarkers in drug discovery and development (Table 2). These include target validation; translating preclinical results rapidly into man; "quick-kill" of agents or classes with poor activity against the targeted pathway; identifying the right subsets of patients for treatment; identifying optimal dose, regime, measurement end-points and inter-subject variability of response; medical personalization (companion diagnostics); and anticipating toxicities or avoiding toxicities through dose-adjustment. Translational markers that are predictive of disease outcomes also allow the selection of animal models that best reflect human disease, or the de-emphasis or even gradual elimination of animal models from the drug-development process.

Table 2. Applications of Causal Pathway Metrics

Less guessing about:

1. Picking targets
2. Choosing chemical class and best compound in class
3. Identifying the right patients (excluding nonresponders subsets at risk for toxicities)
4. Finding the best dose and regimen for clinical trials
5. Selecting intermediate end-points to measure and variability to expect in patients
6. Dosing to avoid minimize toxicities
7. Testing whether personalization can improve response
8. Deciding whether to get out early (quick kill)

Stable Isotope-Based Kinetic Biomarkers Have Advantages over but Are Complementary to Static Biomarkers

Traditional static biomarkers provide information about the concentration, presence or structure of molecules in a living system. In contrast, kinetic biomarkers reveal the dynamic behavior of the pathways that lead to and from these molecules. The amount of collagen in a tissue, for example, does not reveal the rate at which collagen is being synthesized (fibrogenesis) in a disease setting or after starting a therapeutic intervention. Nor does the content of mitochondrial proteins tell us the degree to which mitochondrial biogenesis or mitophagy was induced by an intervention. Similarly, the concentration of a protein in the cerebrospinal fluid does inform us the efficiency at which neurons in the brain

(continued)

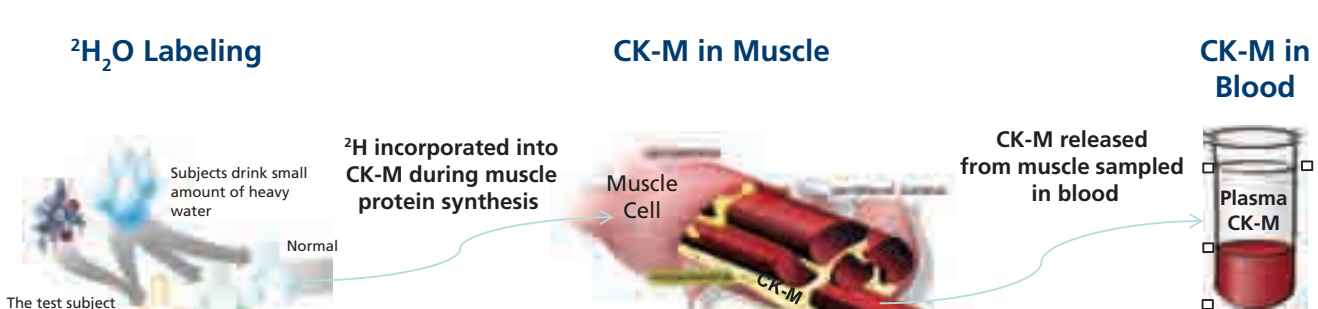


Figure 4. "Virtual Biopsy" Technique for Kinetic Biomarkers. Example of Skeletal Muscle Protein Synthesis from Plasma Creatine Kinase M-type (CK-M).

transported this molecule through axons to nerve terminals. These latter processes all involve, at their core, the flux of molecules through often complex pathways and networks.

The activity of these pathogenic processes or disease pathways are in principle the metrics most closely related to the initiation, severity, progression and therapeutic reversal of a disease. The only way to measure molecular flux rates is by the introduction of isotopic labels, as noted above. Although static parameters can provide key complementary information, such as pool size and net gain or loss of a molecular component, the functional activity of underlying pathogenic processes can only be revealed through kinetic measurements.

The same considerations apply to "Network Dynamics," such as dynamic proteomics, when compared to static "-omics" biomarkers, but with an additional point that is worth noting. Protein synthesis and breakdown rates typically represent a pro-active decision by a cell or organism that is functionally interpretable in context of health or disease. By way of example for proteins, ubiquitin-proteosome-based removal, transcription factor-stimulated synthesis, assembly during biogenesis of an organelle, packaging and secretion in vesicles, modulation through the unfolded protein response, deposition as extracellular matrix, induction as part of a protein signaling cascade, *etc.* – these can all be thought about in functional terms by physiologists, toxicologists and clinicians. The same cannot always be said for the simple presence or concentration of a protein. Because of this marriage between intrinsic functional significance and broad, hypotheses-free screening, dynamic proteomics is a particularly powerful technology for biomarker and target discovery.

Summary and Conclusions

In summary, the recent addition of stable isotope-based biomarkers to the diagnostic repertoire has brought a new and rapidly expanding dimension to drug development. These biomarkers provide functionally interpretable, decision-relevant information about the underlying biology of disease, capturing the activity of causal pathways that are the driving forces underlying disease and therapy. Kinetic biomarkers thereby predict clinical response and its relation to target engagement or the effects of a clinical treatment regimen. Stable isotope-based kinetic biomarkers are particularly powerful additions in the emerging era of personalized medicine.

Related Products

Catalog No.	Description
DLM-4-70	Deuterium oxide (D, 70%)
CLM-1396	D-Glucose (U-$^{13}C_6$, 99%)
CLM-1822-H	L-Glutamine ($^{13}C_5$, 99%)
NLM-202	Glycine (^{15}N, 98%)
CLM-2262-H	L-Leucine ($^{13}C_6$, 99%)
NLM-8401	Spirulina Whole Cells (lyophilized powder) (U-^{15}N, 98%+)

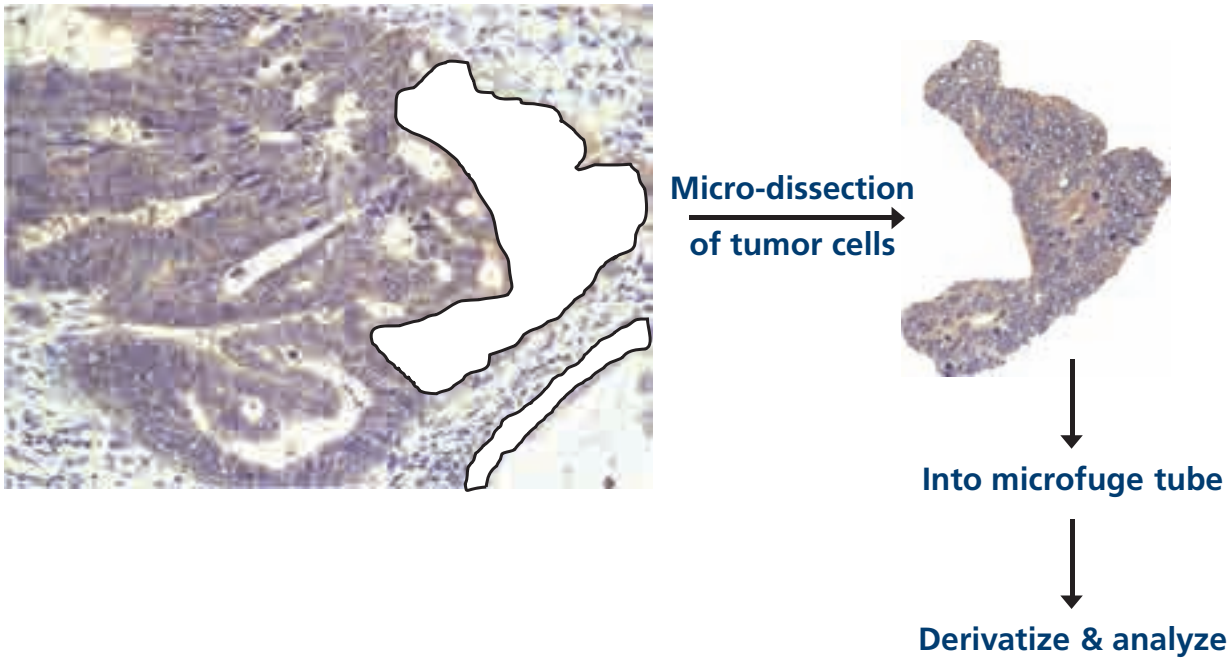


Figure 5. Micro-dissection of Normal and Tumor Tissues for Mass Spectrometric Kinetic Analysis.

References

1. Swann, J.P. **2011**. Summary of NDA Approvals & Receipts, 1938 to the present, FDA History Office, www.fda.gov/AboutFDA/WhatWeDo/History.
2. Duyk, G. **2003**. Attrition and translation. *Science*, *302*, 603-5.
3. Biotechnology Industry Organization (BIO) analysis, **2012**.
4. FDA, Innovation or Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products. **March 2004**.
5. Hellerstein, M.K. **2008**. A critique of the molecular target-based drug discovery paradigm based on principles of metabolic control: advantages of pathway-based discovery. *Metab Eng, 10*, 1-9.
6. Hellerstein, M.K. **2003**. *In vivo* measurement of fluxes through metabolic pathways: the missing link in functional genomics and pharmaceutical research. *Annu Rev Nutr*, *23,* 379-402.
7. Turner, S.M.; Hellerstein, M.K. **2005**. Emerging applications of kinetic biomarkers in preclinical and clinical drug development. *Curr Opin Drug Discov Devel, 8*, 115-26.
8. Hellerstein, M.K. **2008**. Exploiting complexity and the robustness of network architecture for drug discovery. *J Pharmacol Exp Ther, 325*, 1-9;
9. Shankaran, M.; King, C.; Lee, J.; Busch, R.; Wolff, M.; Hellerstein, M.K. **2006**. Discovery of novel hippocampal neurogenic agents by using an *in vivo* stable isotope labeling technique. *J Pharmacol Exp Ther, 319*, 1172-81
10. Fanara, P.; Wong, P.Y.; Husted, K.H.; Liu, S.; Liu, V.M.; Kohlstaedt, L.A.; Riiff, T.; Protasio, J.C.; Boban, D.; Killion, S.; Killian, M.; Epling, L.; Sinclair, E.; Peterson, J.; Price, R.W.; Cabin, D.E.; Nussbaum, R.L.; Brühmann, J.; Brandt, R.; Christine, C.W.; Aminoff, M.J.; Hellerstein, M.K. **2012**. Cerebrospinal fluid-based kinetic biomarkers of axonal transport in monitoring neurodegeneration, *J Clin Invest, 122*, 3159-69.
11. Potter, W.Z. **2012**. Mining the secrets of the CSF: developing biomarkers of neurodegeneration. *J Clin Invest, 122*, 3051-3.
12. Price, J.C.; Khambatta, C.F.; Li, K.W.; Bruss, M.D.; Shankaran, M.; Dalidd, M.; Floreani, N.A.; Roberts, L.S.; Turner, S.M.; Holmes, W.E.; Hellerstein, M.K. **2012**. The effect of long term calorie restriction on *in vivo* hepatic proteostatis: a novel combination of dynamic and quantitative proteomics. *Mol Cell Proteomics, 11*, 1801-14.
13. Price, J.C.; Holmes, W.E.; Li, K.W.; Floreani, N.A.; Neese, R.A.; Turner, S.M.; Hellerstein, M.K. **2012**. Measurement of human plasma proteome dynamics with $^{2}H_{2}O$ and liquid chromatography tandem mass spectrometry. *Anal Biochem, 420*, 73-83.
14. Hayes, G.M.; Simko, J.; Holochwost, D.; Kuchinsky, K.; Busch, R.; Misell, L.; Murphy, E.J.; Carroll, P.; Chan, J.; Shinohara, K.; Hellerstein, M.K. **2012**. Regional cell proliferation in microdissected human prostate specimens after heavy water labeling *in vivo*: correlation with prostate epithelial cells isolated from seminal fluid. *Clin Cancer Res, 18*, 3250-60.
15. Northen, T.; Bowen, B.; Hellerstein, M.K. **2013**. *Nature Techniques* (in press).

More information is available at www.kinemed.com.

Biological Standards

Information obtained in clinical diagnostics requires a level of confidence that is much higher than most other fields due to the implications of the results. They can be indicative of a disease state, a chronic illness, the effects of a drug or a substance in the body. For this reason, the staffing, instrumentation, reagents and supplies in these labs are of the highest quality.

CIL offers a wide variety of high-quality isotope-labeled reagents that are routinely used as reference standards in both research and diagnostic settings. These products are manufactured to meet high quality control specifications for both isotopic enrichment and chemical purity, which are invaluable to accurate and precise results as required by clinical labs. As researchers in the clinical field search for faster, more accurate tests, they are often driven toward mass spectrometry. The use of stable isotopes combined with this technology is emerging as one of the most powerful ways to increase throughput and accuracy in clinical testing.

Vitamins

Vitamins are essential to maintaining the health of an individual. Some are produced endogenously and some are obtained only through one's diet. Certain levels of each vitamin are required for the function of critical organs and the metabolism of carbohydrates, fats and proteins. Vitamin deficiencies can have detrimental impacts on a number of body processes and, therefore, levels often need to be tested. Historically, immunoassays have been used to determine vitamin levels, however, the accuracy of these tests is often questioned. Recently, the powerful combination of mass spectrometry and stable isotope-labeled internal standards has proven to be one of the most accurate ways to identify and quantitate vitamins in even a low-volume sample.

These tests are becoming more robust, reproducible and accurate as the sensitivity of instrumentation and the availability of internal standards both increase. CIL's recently expanded product listing of both carbon-13 and deuterium-labeled vitamins will assist in this effort. Please see pages 161-162 for a complete listing of these vitamins.

Vitamin D

Responsible for absorption of calcium and phosphate, Vitamin D is one of the most important organic chemical compounds in the body as it promotes healthy growth and bone repair. It is synthesized endogenously by most mammals when they are exposed to sunlight and is also supplemented through diet. A deficiency in Vitamin D can lead to osteomalacia, which is the softening of the bones. Though it is most prevalent in older individuals, it can occur at any age. Biomarkers for vitamin D deficiency can be identified in plasma samples utilizing mass spectrometry and stable isotope-labeled internal standards. CIL offers a wide variety of labeled vitamin D and vitamin D metabolites that can be used in these tests, some which are also available as carbon-13 labeled for ease of use with LC/MS.

Catalog No.	Description
DLM-9105	1,25-Dihydroxyvitamin D_2 (6,19,19-D_3, 99%) CP 95%
DLM-9107	1,25-Dihydroxyvitamin D_3 (6,19,19-D_3, 97%) CP 95%
DLM-9111	3-*epi*-25-Hydroxyvitamin D_3 (6,19,19-D_3, 98%)
CLM-9113	25-Hydroxyvitamin D_2 (25,26,27-$^{13}C_3$, 99%)
DLM-9114	25-Hydroxyvitamin D_2 (6,19,19-D_3, 97%)
DLM-9116	25-Hydroxyvitamin D_3 (6,19,19-D_3, 97%)
DLM-7708	25-Hydroxyvitamin D_3 (26,26,26,27,27,27-D_6, 98%)
DLM-8985	Vitamin D_2 (Ergocalciferol) (6,19,19-D_3, 97%)
CLM-7850	Vitamin D_3 (Cholecalciferol) ($^{13}C_2$, 99%) CP 90%

"Quantitative analysis in clinical diagnostics using mass spectrometry remains a difficult endeavor particularly for small molecules due to chemical similarity and isobaric forms of many substances. Both chromatography and the use of isotopically labeled internal standards to perform small molecule quantification are required to obtain good quantitative results in many applications. The use of isotopically labeled internal standards remains the best solution as these standards ideally match the chemical behavior of their analytes, thus leading to better quantification than obtained when using structure homologues with physicochemical characteristics."

Assoc. Prof. Dipl.-Ing. DDr. David C. Kasper
Rummelhardtgasse 3/38, 1090 Wien
Österreich

Biological Standards

Steroids

The use of anabolic steroids among athletes to enhance performance in sports has become an increasingly large problem over the past few decades. These athletes are routinely tested by agencies such as the World Anti-Doping Agency, to determine levels of steroids present in their bodies. Many of these tests involve taking samples of serum or urine and spiking in an internal standard or a combination of internal standards to quantitate even low levels of a given steroid or group of steroids.

CIL is proud to offer highly enriched stable-isotope labeled steroids to assist with ease of quantification in these critical tests. Below are the most widely used steroids. **Please see pages 158-159 for a complete listing.**

Catalog No.	Description
DLM-8438	Aldosterone (2,2,4,6,6,17,21,21-D_8)
DLM-9137	Androsterone glucuronide (2,2,4,4-D_4, 98%)
DLM-6780	Chenodeoxycholic acid (2,2,4,4-D_4, 98%)
DLM-7347	Corticosterone (2,2,4,6,6,17α,21,21-D_8, 97-98%)
DLM-2057	Cortisol (9,12,12-D_3, 98%)
DLM-2218	Cortisol (9,11,12,12-D_4, 98%)
DLM-7209	11-Deoxycortisol (21,21-D_2, 96%)
DLM-8305	21-Deoxycortisol (D_8, 96%)
DLM-170	Diethylstilbestrol (*cis*/*trans* mix) (ring-3,3′,5,5′-diethyl-1,1,1′,1′-D_8, 98%)
DLM-2487	Estradiol (2,4,16,16-D_4, 95-97%)
DLM-3976	Estrone (2,4,16,16-D_4, 97%)
DLM-4691	17α-Ethynylestradiol (2,4,16,16-D_4, 97-98%)
DLM-6598	17α-Hydroxyprogesterone (2,2,4,6,6,21,21,21-D_8, 98%)
CLM-2468	Norethindrone (ethynyl-$^{13}C_2$, 99%)
DLM-3754	5-α-Pregnan-3-α-ol-20-one (17,21,21,21-D_4, 96-98%) CP 95%+
DLM-6896	Pregnenolone (17,21,21,21-D_4, 98%)
CLM-159	Testosterone (3,4-$^{13}C_2$, 99%)
CLM-9164	Testosterone (2,3,4-$^{13}C_3$, 99%)

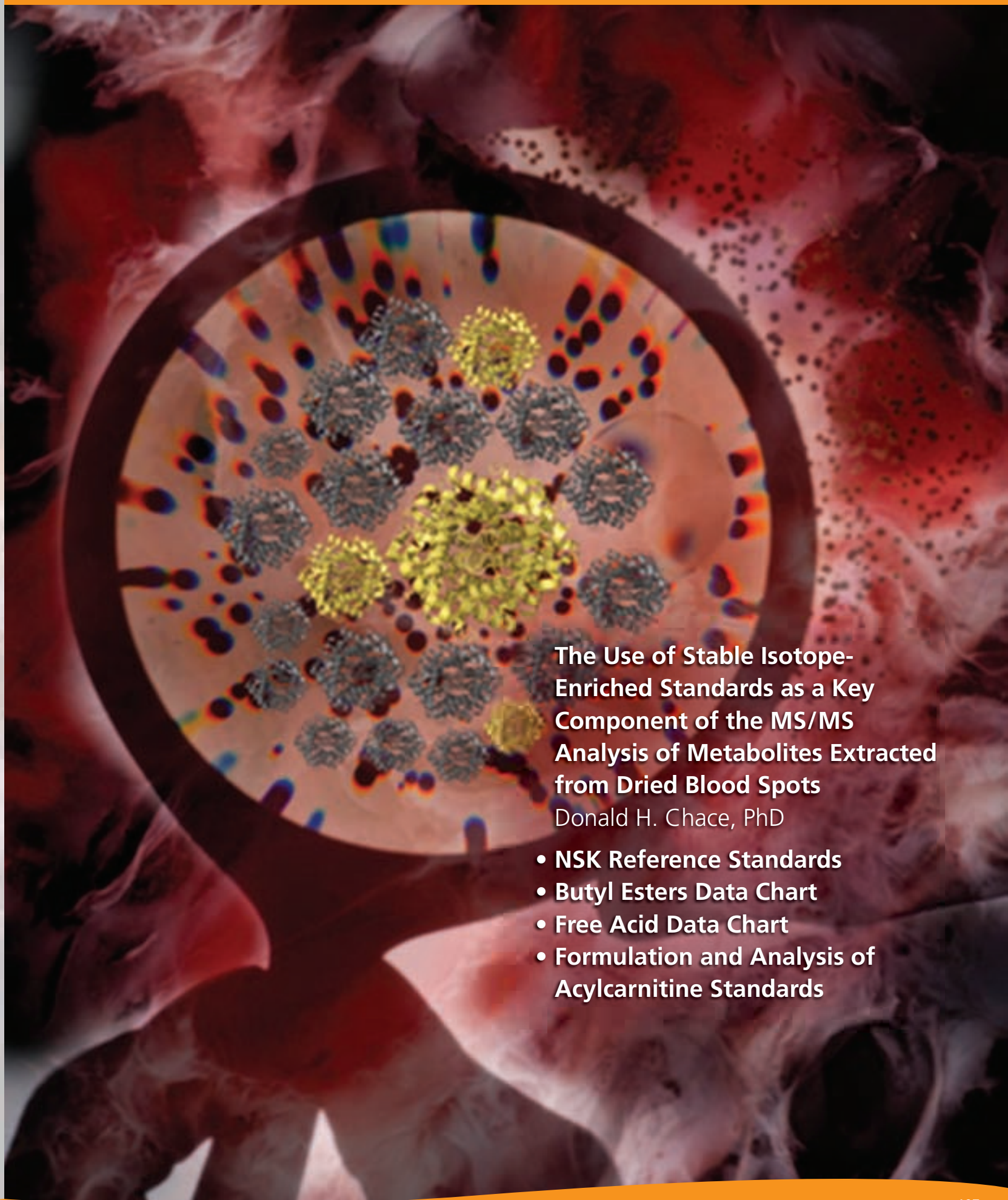
Cholesterol

Cholesterol is essential to mammals for many reasons. Not only is it an important structural component on the cellular level, specifically in the cell membrane, it is also a precursor for many vital biochemicals in the body. Cholesterol is endogenously produced within mammals and is also obtained through diet, with higher concentrations in animal fats. When cholesterol is obtained through diet, endogenous production often slows to moderate overall cholesterol levels as abnormal levels can be detrimental to the health of an individual and are associated with heart disease, stroke and diabetes. Isotope-labeled forms of cholesterol allow for quantitative analysis in plasma samples to quantitate cholesterol levels as well as *in vivo* analysis in mammals to better understand cholesterol synthesis and degradation.

Catalog No.	Description
CLM-9139	Cholesterol (2,3,4-$^{13}C_3$, 99%)
CLM-804	Cholesterol (3,4-$^{13}C_2$, 99%)
DLM-1831	Cholesterol (3-D_1, 98%)
DLM-7260	Cholesterol (25,26,26,26-D_4, 98%)
DLM-2607	Cholesterol (2,2,3,4,4,6-D_6, 97-98%)
DLM-3057	Cholesterol (25,26,26,26,27,27,27-D_7, 98%)
OLM-7695	Cholesterol (^{18}O, 80%)
CLM-3361	Cholesterol-3-octanoate (octanoate-1-^{13}C, 99%)

"Stable isotopes, together with mass spectrometry, provide the clinical chemist with the tools to develop and utilize the most accurate and precise laboratory tests possible in research, screening and diagnostics. I have worked with CIL for more than 15 years to design and provide the best and most suitable stable isotope standards for the newborn and metabolic screening community. Their standards are the highest quality and customer service is excellent."

Donald H. Chace, PhD, MSFS, FACB
The Pediatrix Center for Research, Education and Quality
Pediatrix Medical Group



The Use of Stable Isotope-Enriched Standards as a Key Component of the MS/MS Analysis of Metabolites Extracted from Dried Blood Spots
Donald H. Chace, PhD

- **NSK Reference Standards**
- **Butyl Esters Data Chart**
- **Free Acid Data Chart**
- **Formulation and Analysis of Acylcarnitine Standards**

The Use of Stable Isotope-Enriched Standards as a Key Component of the MS/MS Analysis of Metabolites Extracted from Dried Blood Spots

Donald H. Chace, MSFS, PhD, FACB
Pediatrix Analytical, Pediatrix Center for Research, Education and Quality
Pediatrix Medical Group, Sunrise, FL 33323 USA

Isotopes and Clinical Analysis

Accurate quantification of endogenous and exogenous metabolites and biomarkers of disease is essential to laboratory medicine and clinical research. The methods chosen have to meet the analytical criteria of high sensitivity (low detection limits), high selectivity (few interferences from compounds not being measured) and excellent precision (reproducibility). Immunoassays are the foundation of hospital-based clinical analysis and a major part of commercial diagnostic and newborn screening laboratories. These methods have the advantage of being inexpensive, easy to use, supported technically, sensitive and able to offer a wide scope of analytical targets. Their main drawback is poorer selectivity compared to newer technology such as mass spectrometry. Mass spectrometric (MS) based methods are more superior in their selectivity because they are based on the detection of chemical and physical characteristics related to its elemental composition and structure. MS methods have adequate analytical sensitivity for most applications and can be quite precise especially if stable isotopes are utilized as reference standards. Addition of stable isotope-labeled internal standards to a biological specimen is commonly referred to as isotope dilution mass spectrometry (IDMS). The basis for IDMS is that a known amount of the analyte to be measured that has been enriched with one or more isotopes (*i.e.* deuterium, carbon-13) is added to (or diluted with) its unlabeled target analyte. The mass spectrometer measures each analyte separately based on their mass-to-charge ratios, and a concentration can be based on the quantity of the labeled and unlabeled compound detected.

Dried Blood Spots and Metabolic Screening

One area of clinical laboratory science that has gained much attention in recent years is metabolic screening or newborn screening. 2013 marks the 50th anniversary of the introduction of analyzing blood for metabolites that are key markers for inherited disease. In 1960, Dr. Robert Guthrie developed a method for measuring phenylalanine (Phe) in the blood of infants to detect whether an infant was at risk for phenylketonuria (PKU), a disorder of Phe metabolism. Early detection of this disease was shown to reduce the mental retardation in affected infants by enabling early intervention and treatment, often as simple as a dietary change. However, rather than utilize a liquid specimen (whole blood or plasma) which would require expensive shipping from every birthplace, Dr. Guthrie championed the use of collecting blood on a strip of filter paper, drying the specimen and sending by regular mail. In addition to cost savings, blood dried on filter paper has a small footprint for storage, is less infectious and requires smaller volumes of blood collected from infants (~1/10th). During the decades that followed Guthrie's PKU test, newer analytical methods were developed to detect PKU and an array of other disorders of hormone, amino acid and carbohydrate metabolism.

Metabolic screening programs have had one great concern that is always an important topic: accuracy in measuring abnormal concentrations of metabolites in a dried blood spot (DBS) as it relates to blood volume in a sample. Hematocrit, the volume of blood applied to the filter paper, and the absorption characteristics of filter paper are critical in the quantification of metabolites. Although the manufacturing of paper and its absorptive characteristics could be controlled, the hematocrit and manner and volume of blood applied is much less controlled. Add patient metabolic variability to the volume variations and you have an analysis that is less precise than its liquid counterpart.

MS/MS and Stable Isotope Internal Standards

Tandem mass spectrometry (MS/MS) is a specific type of MS method that has two mass analyzers separated by a fragmentation chamber (collision cell) that can break ionized molecules (precursor ions, intact molecular ions) into specific and reproducible and smaller pieces or product ions. Amino acids and acylcarnitines as classes of compounds produce common highly reproducible and charcterizable fragments. Using MS/MS one can selectively detect just acylcarnitines or alpha amino acids in separate scans, simultaneously, without any chromatography yet still maintain high selectivity. Therefore, MS/MS can detect many different compounds in a single analysis in about two minutes per sample. It is for this reason that MS/MS has replaced older methods for amino acid analysis and detection of metabolites such as phenylalanine for PKU and has added a series of metabolites, *i.e.* the acylcarnitines for a series of disorders such as MCAD (medium chain acyl CoA dehydrogenase deficiency). In total, several dozen metabolites are detected in a single analysis.

Previous page: artwork by Surinova Silva.

Quantification is critical to MS/MS analysis and therefore requires reference standards to properly measure the concentration of the markers.

The ideal reference standards for mass spectrometry are stable isotope-enriched analogues of the most important screening markers such as phenylalanine (Phe) or octanoylcarnitine (C8).

The design of internal standards in terms of choice of isotope, the number of isotopes, their position in the molecule, high purity and high enrichment is critical to MS/MS analysis. During a period of two decades, standards have been introduced to meet the quantitative requirements of MS/MS analysis in dried blood spots for amino acids and acylcarnitines. For example, amino acids primarily lose a formic acid molecule (or formic acid butyl ester if derivatized) in the MS/MS analysis. In order to detect and quantify the precursor ion (original ionized molecule) in both labeled and native forms, the carboxyl carbon cannot be enriched with ^{13}C. Further, a minimum of two (preferably three or more) enriched atoms must be achieved in order to shift the mass sufficiently from its non-enriched value. Finally the choice of isotope must be such that if deuterium, it is in a non-exchangeable position, *i.e.* $^{13}C_6$ is used for the isotope of phenylalanine.

Enriched Sets of Common Metabolites Used in DBS Analysis

Historically, just a few key internal standards were produced in a few hundred milligram quantities. Amino acid stable isotope-enriched standards were often readily available, but acylcarnitines generally required special synthesis from specialty labs. Acylcarnitines are also notoriously unstable in the long term, especially in solution, and it has been difficult to always ensure adequate amounts of good-quality standards. Most importantly, due to their relatively high expense and limited quantity, the preparation of standards was a challenge when just a few milligrams were prepared. For all of these reasons and to increase the number and variety of standards to meet the requirements of a complex comprehensive MS/MS profile, more than 20 standards were synthesized. In my experience, weighing out small quantities of standards (2-3 mg or less) was a challenge especially as some standards are hygroscopic. Weighing out 200-300 mg was not practical because those quantities were not available for many standards or were cost-prohibitive. Instability added to problems since solutions of acylcarnitines in methanol hydrolyzed in less than two weeks, producing lower concentrations of short-chain acylcarnitines and higher concentrations of free carnitine. The solution was obtaining all of the standards required for our MS/MS analytical needs for DBS analysis in two or three sets and individual vials with dry standard such that vials could be reconstituted as needed.

Current State of the Art

There are over 20 acylcarnitines and amino acid stable isotope-enriched standards used in metabolic screening and research in dried blood spots. These standards can be obtained in three sets of standards available from CIL (NSK-A, NSK-B, NSK-B-G). The amount used per day in a laboratory primarily depends on the screening volume (samples/day), size of the dried blood spot sample (1/8th or 3/16th), and the relative amount of standard desired relative to the extracted endogenous metabolite. The internal standards are reconstituted in pure methanol (acylcarnitines) or a 50/50 methanol/water mix (amino acids). They can then be mixed together and diluted with pure methanol to the concentration needed. In each assay a specific volume of the of the methanol containing the stable isotope internal standards is mixed with the blood spot. Only the extracted metabolites can be quantified with the internal standard in the methanol mixture. However, in every step that follows, the internal standard and its unlabeled isotope analog is carried out in a manner consistent with traditional IDMS methods. For DBS, therefore, we designate it as pseudo IDMS because a quantitative error may be introduced if the extraction efficiency is much less than 100%. Fortunately for most acylcarnitines and amino acids, the extraction efficiency is greater than 90%. The method is validated with various peer-reviewed publications and in fact, a large number of metabolic screening labs have embraced MS/MS technology and have chosen to use pre-prepared sets of standards for improved reliability and more accurate quantification.

Future Applications

The use of stable isotope standards is not limited to the analysis of dried blood spots for newborn and metabolic screening. In fact, the use of isotope standards suitable to the analyte being measured are now being investigated for use in drug metabolism and pharmacokinetic studies. In addition, new dried matrices of biological fluids (*i.e.* plasma, urine) are being investigated with unique applications of stable isotope standards for quantification, *i.e.* fluids or filter paper pre-spiked with stable isotope standards) that better approximate traditional IDMS. Clearly, research using stable isotopes, mass spectrometry and dried blood specimens has much growth ahead, far beyond newborn screening. It's only a matter of time before other industries and scientific fields like environmental science, forensic science and materials science use dried specimens and stable isotope standards in new, unique and cost-effective ways.

NSK-A – Amino Acid Reference Standards

This set contains ten vials of a dry mixture of 12 isotopically labeled amino acids. Accurate and complete reconstitution of the contents of one vial in 1 mL of high purity solvent will produce the concentrations presented in the Standards Concentrations table. Mix well. This solution becomes the concentrated amino acid stock standard.

Dilution of Reference Standards Concentrated Working Stock

To prepare working stock solutions, one of the following procedures is suggested: dilute 1 mL (reconstituted vial contents per instructions above) of the concentrated amino acid stock standard with pure solvent. If Set B (Acylcarnitine Reference Standards) was purchased, mix 1 mL (reconstituted vial contents) of concentrated standards from Set A with 1 mL of the concentrated standards from Set B. Store the diluted standards in a tightly sealed vial at 4°C. In order to maintain the integrity of the solution, we recommend storing the sealed vials in a second sealed container. We recommend discarding this concentrated working stock solution after ~1 month. Stability data is being obtained.

Standards Concentrations

Reference Standard	Concentration (nmol/mL)
Glycine (2-^{13}C; ^{15}N)	2500
L-Alanine (2,3,3,3-D_4)	500
L-Valine (D_8)	500
L-Leucine (5,5,5-D_3)	500
L-Methionine (methyl-D_3)	500
L-Phenylalanine (ring-$^{13}C_6$)	500
L-Tyrosine (ring-$^{13}C_6$)	500
L-Aspartic acid (2,3,3-D_3)	500
DL-Glutamic acid (2,4,4-D_3)	500
L-Ornithine•HCl (5,5-D_2)	500
L-Citrulline (5,5-D_2)	500
L-Arginine•HCl (5-^{13}C; 4,4,5,5-D_4)	500

NSK-B – Free Carnitine and Acylcarnitine Reference Standards

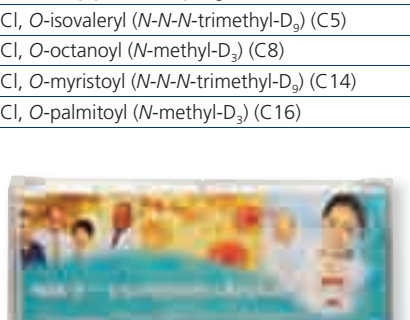
This set contains ten vials of a dry mixture of eight isotopically labeled free carnitine and acylcarnitines. Accurate and complete reconstitution of the contents of one vial in 1 mL of high purity solvent will produce the concentrations presented in the Standards Concentrations table. Mix well. This solution becomes the concentrated acylcarnitine stock standard.

Dilution of Reference Standards Concentrated Working Stock

To prepare working stock solutions, one of the following procedures is suggested: dilute 1 mL (reconstituted vial contents per instructions above) of the concentrated acylcarnitine stock standard with pure solvent. If Set A (Amino Acid Reference Standards) was purchased, mix 1 mL (vial contents) of concentrated standards from Set A with 1 mL of the concentrated standards from Set B. Store the diluted standards in a tightly sealed vial at 4°C. In order to maintain the integrity of the solution, we recommend storing the sealed vials in a second sealed container. We recommend discarding this concentrated working stock solution after ~1 month. Stability data is being obtained.

Standards Concentrations

Reference Standard	Concentration (nmol/mL)
L-Carnitine (trimethyl-D_9) (free carnitine, CN)	152.0
L-Carnitine•HCl, *O*-acetyl (*N*-methyl-D_3) (C2)	38.0
L-Carnitine•HCl, *O*-propionyl (*N*-methyl-D_3) (C3)	7.6
L-Carnitine•HCl, *O*-butyryl (*N*-methyl-D_3) (C4)	7.6
L-Carnitine•HCl, *O*-isovaleryl (*N*-*N*-*N*-trimethyl-D_9) (C5)	7.6
L-Carnitine•HCl, *O*-octanoyl (*N*-methyl-D_3) (C8)	7.6
L-Carnitine•HCl, *O*-myristoyl (*N*-*N*-*N*-trimethyl-D_9) (C14)	7.6
L-Carnitine•HCl, *O*-palmitoyl (*N*-methyl-D_3) (C16)	15.2



NSK-B-G – Supplemental Acylcarnitine Reference Standards

This set contains 10 vials of a dry mixture of four isotopically labeled acylcarnitines. Accurate and complete reconstitution of the contents of one vial in 1 mL of high purity solvent will produce the concentrations presented in the Standards Concentrations table. Mix well. This solution becomes the Concentrated Supplemental Acylcarnitines Stock Standard.

Dilution of Reference Standards Concentrated Working Stock

To prepare working stock solutions, mix 1 mL (vial contents) of concentrated standards from NSK-A with 1 mL of the concentrated standards from NSK-B and 1 mL of the concentrated standards from NSK-B-G. Store the diluted standards in a tightly sealed vial at 4°C. In order to maintain the integrity of the solution, we recommend storing the sealed vials in a second sealed container. We recommend discarding this concentrated working stock solution after ~one month. Stability data is being obtained.

Standards Concentrations

Reference Standard	Concentration (nmol/mL)
L-Carnitine (mono)•ClO_4, *O*-glutaryl (*N*-methyl-D_3) (C5DC)	15.20
L-Carnitine•ClO_4, 3-hydroxyisovaleryl (*N*-methyl-D_3) (C5OH)	7.60
L-Carnitine•HCl, *O*-dodecanoyl (*N*-*N*-*N*-trimethyl-D_9) (C12)	7.60
L-Carnitine•HCl, *O*-octadecanoyl (*N*-methyl-D_3) (C18)	15.20

NSK-T – Succinylacetone Reference Standards

This set contains 10 vials of isotopically labeled succinylacetone. Accurate and complete reconstitution of the contents of one vial in 1 mL of high purity solvent will produce the concentrations presented in the Standards Concentrations table. Mix well. This solution becomes the Concentrated Succinylacetone Stock Standard.

Dilution of Reference Standards Concentrated Working Stock

To prepare working stock solutions, the following procedure is suggested: dilute 1 mL (reconstituted vial contents per instructions above) of the concentrated succinylacetone standard with pure solvent. Store the diluted standard in a tightly sealed vial at 4°C. In order to maintain the integrity of the solution, we recommend storing the sealed vial in a second sealed container. We recommend discarding this concentrated working stock solution after ~1 month. Stability data is being obtained.

Standards Concentrations

Reference Standard	Concentration (nmol/mL)
Succinylacetone (3,4,5,6,7-$^{13}C_5$)	1000

Butyl Esters Data Chart

Neutral and Acidic Amino Acids (NSK-A)			
m/z	Compound	Abbr.	*Comments (NL 102)*
132	Glycine	Gly	
134	***Glycine**	***Gly**	**$^{13}C^{15}N$**
146	Alanine	Ala	
150	***Alanine**	***Ala**	**D_4**
162	Serine	Ser	
172	Proline	Pro	
174	Valine	Val	
176	Threonine	Thr	
182	***Valine**	***Val**	**D_8**
186	Glutamine	Gln	(Glu – NH_3)
188	Leucine+	Leu+	Isoleucine, HydroxyProline, Allo-Ile
191	***Leucine**	***Leu**	**D_3**
206	Methionine	Met	
209	***Methionine**	***Met**	**D_3**
212	Histidine	His	
222	Phenylalanine	Phe	
228	***Phenylalanine**	***Phe**	**$^{13}C_6$**
238	Tyrosine	Tyr	
244	***Tyrosine**	***Tyr**	**$^{13}C_6$**
246	Aspartic Acid	Asp	
249	***Aspartic Acid**	***Asp**	**D_3**
260	Glutamic Acid	Glu	
263	***Glutamic Acid**	***Glu**	**D_3**

Basic Amino Acids (NSK-A)			
m/z	Compound	Abbr.	*Comments*
189	Ornithine	Orn	NL 119
191	***Ornithine**	***Orn**	**D_2**
232	Citrulline	Cit	NL 119
234	***Citrulline**	***Cit**	**D_2**
231	Arginine	Arg	NL 161
236	***Arginine**	***Arg**	**$D_4{}^{13}C$**

NL = Neutral Loss

Legend: NSK-A = blue, NSK-B = green, NSK-B-G = red
For Research Use Only. Not for diagnostic procedures.

Free Carnitine (NSK-B)			
m/z	Compound	Abbr.	*Comments (Pre 85)*
218	Free Carnitine	C0, FC	Pre 85 and Pre 103
221	*Hydro-Free Carnitine	*Hydro-FC	Hydrolyzed D_3 AC STDS
227	***Free Carnitine**	***FC**	**D_9**

Acylcarnitines (NSK-B, NSK-B-G)			
m/z	Compound	Abbr.	*Comments*
260	Acetyl-	C2	(+ glutamic acid)
263	***Acetyl-**	***C2**	**D_3 (+ D_3-Glu)**
274	Propionyl-	C3	
277	***Propionyl-**	***C3**	**D_3**
288	Butyryl-	C4	
291	***Butyryl-**	***C4**	**D_3**
300	Tiglyl-	C5:1	
302	Isovaleryl-	C5	Methylbutyryl-
304	Hydroxybutyryl-	C4OH	
311	***Isovaleryl-**	***C5**	**D_9**
316	Hexanoyl-	C6	
318	Hydroxyisovaleryl-	C5OH	
321	***Hydroxyisovaleryl-**	***C5OH**	**D_3**
344	Octanoyl-	C8	
347	***Octanoyl-**	***C8**	**D_3**
360	Malonyl-	C3DC	
368	Decadienoyl-	C10:2	
370	Decenoyl-	C10:1	
372	Decanoyl-	C10	
374	Methylmalonyl-	C4DC	
388	Glutaryl-	C5DC	
391	***Glutaryl**	***C5DC**	**D_3**
400	Dodecanoyl-	C12	
409	***Dodecanoyl**	***C12**	**D_9**
426	Tetradecenoyl-	C14:1	
428	Tetradecanoyl-	C14	
437	***Tetradecanoyl-**	***C14**	**D_9**
456	Palmitoyl-	C16	
459	***Palmitoyl-**	***C16**	**D_3**
472	Hydroxypalmitoyl-	C16OH	
482	Octadecenoyl-	C18:1	
484	Octadecanoyl-	C18	
487	***Octadecanoyl-**	***C18**	**D_3**
498	Hydroxyoctadecenoyl-	C18:1 OH	
500	Hydroxyoctadecanoyl-	C18OH	

Note: Customers can request a laminated copy of this chart.

Free Acid (non-derivatized) Data Chart

Neutral and Acidic Amino Acids (NSK-A)			
m/z	Compound	Abbr.	*Comments (NL 46)*
76	Glycine	Gly	
78	***Glycine**	***Gly**	**$^{13}C^{15}N$**
90	Alanine	Ala	
94	***Alanine**	***Ala**	**D_4**
106	Serine	Ser	
116	Proline	Pro	
118	Valine	Val	
120	Threonine	Thr	
126	***Valine**	***Val**	**D_8**
130	Glutamine	Gln	(Glu – NH_3)
132	Leucine+	Leu+	Isoleucine, HydroxyProline, Allo-Ile
135	***Leucine**	***Leu**	**D_3**
150	Methionine	Met	
153	***Methionine**	***Met**	**D_3**
156	Histidine	His	
166	Phenylalanine	Phe	
172	***Phenylalanine**	***Phe**	**$^{13}C_6$**
182	Tyrosine	Tyr	
188	***Tyrosine**	***Tyr**	**$^{13}C_6$**
134	Aspartic Acid	Asp	
137	***Aspartic Acid**	***Asp**	**D_3**
148	Glutamic Acid	Glu	
151	***Glutamic Acid**	***Glu**	**D_3**

Basic Amino Acids (NSK-A)			
m/z	Compound	Abbr.	*Comments*
133	Ornithine	Orn	NL 63
135	***Ornithine**	***Orn**	**D_2**
176	Citrulline	Cit	NL 63
178	***Citrulline**	***Cit**	**D_2**
175	Arginine	Arg	NL 105
180	***Arginine**	***Arg**	**$D_4{}^{13}C$**

NL = Neutral Loss

Legend: NSK-A = blue, NSK-B = green, NSK-B-G = red
For Research Use Only. Not for diagnostic procedures.

Free Carnitine (NSK-B)			
m/z	Compound	Abbr.	*Comments (Pre 85)*
162	Free Carnitine	C0, FC	Pre 85 and Pre 103
165	*Hydro-Free Carnitine	*Hydro-FC	Hydrolyzed D_3 AC STDS
171	***Free Carnitine**	***FC**	**D_9**

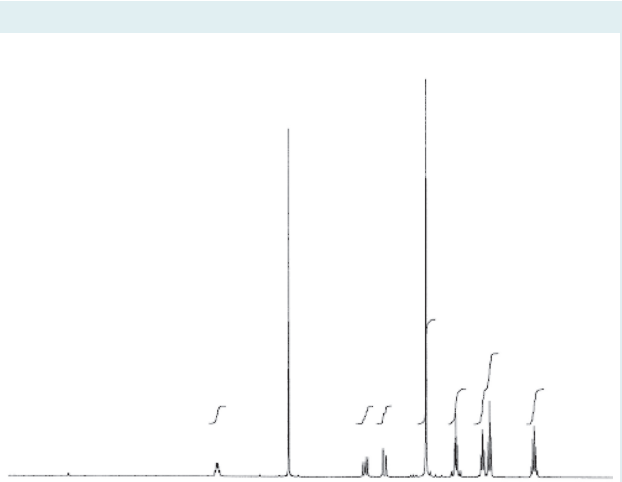
Acylcarnitines (NSK-B, NSK-B-G)			
m/z	Compound	Abbr.	*Comments*
204	Acetyl-	C2	
207	***Acetyl-**	***C2**	**D_3**
218	Propionyl-	C3	
221	***Propionyl-**	***C3**	**D_3**
232	Butyryl-	C4	
235	***Butyryl-**	***C4**	**D_3**
244	Tiglyl-	C5:1	
246	Isovaleryl-	C5	Methylbutyryl-
248	Hydroxybutyryl-	C4OH	Malonyl-
255	***Isovaleryl-**	***C5**	**D_9**
260	Hexanoyl-	C6	
262	Hydroxyisovaleryl-	C5OH	Methylmalonyl-
265	***Hydroxyisovaleryl-**	***C5OH**	**D_3**
288	Octanoyl-	C8	
291	***Octanoyl-**	***C8**	**D_3**
248	Malonyl-	C3DC	Hydroxybutyryl-
312	Decadienoyl-	C10:2	
314	Decenoyl-	C10:1	
316	Decanoyl-	C10	
262	Methylmalonyl-	C4DC	Hydroxyisovaleryl-
276	Glutaryl-	C5DC	
279	***Glutaryl**	***C5DC**	**D_3**
344	Dodecanoyl-	C12	
353	***Dodecanoyl**	***C12**	**D_9**
370	Tetradecenoyl-	C14:1	
372	Tetradecanoyl-	C14	
381	***Tetradecanoyl-**	***C14**	**D_9**
400	Palmitoyl-	C16	
403	***Palmitoyl-**	***C16**	**D_3**
416	Hydroxypalmitoyl-	C16OH	
426	Octadecenoyl-	C18:1	
428	Octadecanoyl-	C18	
431	***Octadecanoyl-**	***C18**	**D_3**
442	Hydroxyoctadecenoyl-	C18:1 OH	
444	Hydroxyoctadecanoyl-	C18OH	

Note: Customers can request a laminated copy of this chart.

Formulation and Analysis of Acylcarnitine Standards

Cambridge Isotope Laboratories, Inc. provides *O*-acylcarnitines of high chemical purity as individual components and kits. As part of this program, CIL offers:

- Straight-chain *O*-acylcarnitines from C0 to C26 in high chemical purity with D_3, D_6, or D_9 labeling.
- Branched-chain and other substituted *O*-acylcarnitines, including glutaryl, isovaleryl, 3-hydroxyisovaleryl, and 2-decenoyl carnitines, also with D_3, D_6, or D_9 labeling.
- High purity unlabeled reference standards corresponding to all labeled analogs.
- Kits prepared under batch record control, analyzed against certified standards with excellent reproducibility and quality assurance.



^{1}H NMR spectrum of *O*-glutaryl-L-carnitine (*N*-methyl-D_3)

Reference Materials

Before isotopically labeled carnitine standard solutions can be formulated and tested, corresponding unlabeled ("native") reference materials must be purified and characterized. We have observed that unlabeled materials available from other manufacturers are often of insufficient purity to use as reference standards. At CIL, we independently synthesize and purify each of these reference materials. The identity and purity of native carnitines are established using quantitative nuclear magnetic resonance (NMR) spectroscopy, high performance liquid chromatography (HPLC) and melting-point determinations. Quantitative NMR is the primary analytical technique, using a common reference material for all the carnitines analyzed.

With pure, well-characterized reference materials in hand, we take similar steps to synthesize, purify and analyze labeled carnitines. Enrichment, the amount of stable isotope incorporation, is measured relative to native analogs by NMR or liquid chromatography-mass spectrometry (LC/MS) techniques. The ^{1}H NMR spectrum of *O*-glutaryl-L-carnitine (*N*-methyl-D_3) is shown above.

Unlabeled Standard Solutions

The gravimetry is traceable to US National Institute of Standards and Technology (NIST) standards. The weights and balances are calibrated on a regular schedule. Class A volumetric glassware is used. These rigorous procedures allow us to control and calculate the uncertainty for concentrations of the unlabeled certified standard solutions, according to EURACHEM/CITAC guidelines.

NSK-B Formulation and Dispensing

Labeled carnitine standard solutions are formulated using similar procedures. Once the concentration of the labeled carnitine solution has been verified against the unlabeled standard (described in detail, below), the solution is metered into vials using a calibrated pipette. The mass of solution added to each vial (and hence the amount of labeled standard) is individually verified. The transfer process is organized into discrete blocks, referred to as "dispenses," to enhance traceability. The solutions in the individual vials are evaporated under vacuum in a carefully controlled environment.

Sampling and Analysis

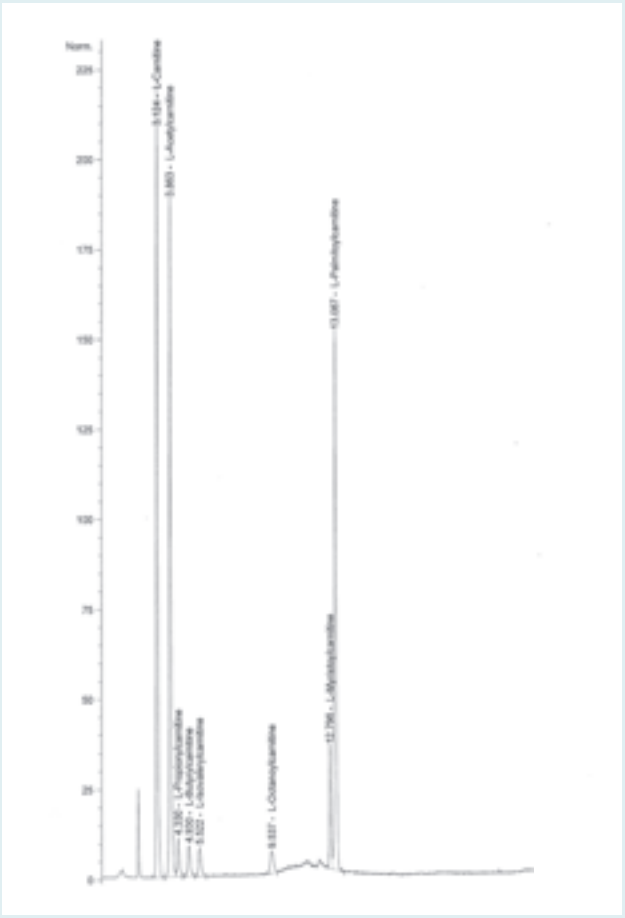
Samples of the finished product are taken to verify the reconstituted concentrations of the carnitines. Quality-control samples are drawn according to American National Standards Institute/American Society for Quality Control (ANSI/ASQC) sampling guidelines.

Certified carnitine standards are formulated at five concentrations, bracketing the target concentrations for the product (0.750x, 0.875x, 1.000x, 1.250x, 1.500x). The carnitines are analyzed by HPLC, using an evaporative light-scattering detector (ELSD), which is sensitive to a wide range of materials, including carnitines, at low concentrations. Other typical HPLC detectors (*e.g.*, ultraviolet, UV, RI) are not sensitive enough to analyze carnitines at the required concentrations. As with many analytical detectors, the response is non-linear. Quadratic or cubic equations are fitted to the calibration curves, with typical correlation coefficients ranging

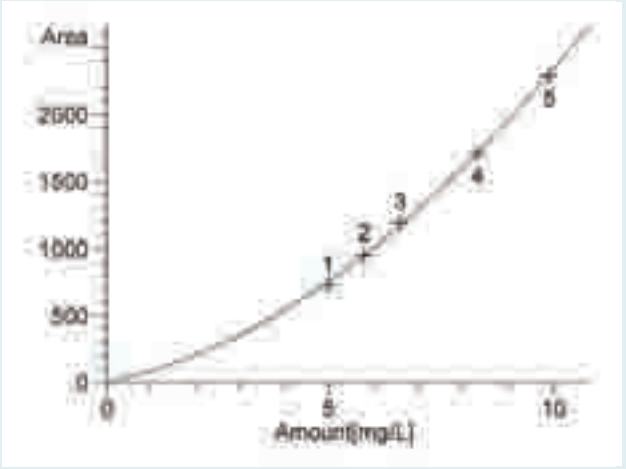
from 0.99995 to 0.99911. Calibration standards are run, interspersed among the product samples with typically five standard concentrations before each set of 5 (or 6) samples.

Calculations and Results

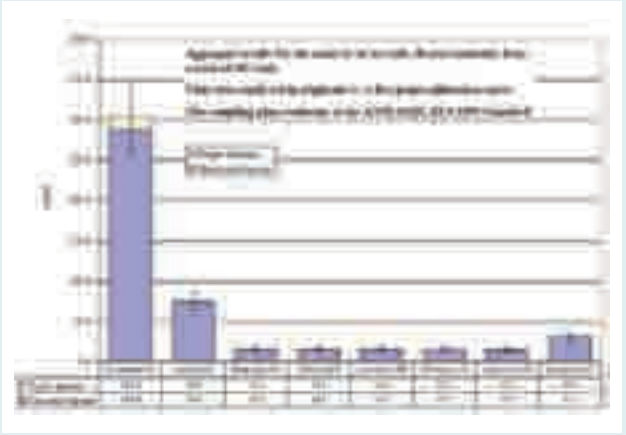
The ELSD measures concentrations by weight (mg/L).
To compare these values to the specification, the concentrations are converted to micro-moles per liter (µM/L). The measured molar concentrations compare well to the corresponding targets. The upper and lower bounds represent the target concentration +/- 15%.



HPLC chromatogram of NSK-B.



Quadratic Calibration Curve for L-Palmitoylcarnitine
Area = 9.992 x (Amount)^2 + 27.31 x (Amount) - 0.476
Correlation: 0.99984



NSK-B-2X, PR-19855 molar concentration compared to specification

- **Environmental Standards**

  Human Biomonitoring: Attogram Level Sensitivity and Consequences for Analytical Standards Purity

  Donald G. Patterson Jr., PhD

- **Perfluorokerosene**
- **Phthalate and Phthalate Metabolite Standards**
- **Prescription and Non-Prescription Drug Standards**
- **Veterinary and Human Antibiotic Standards**
- **Steroids**

Environmental Contaminant Standards for Use in Isotope Dilution Mass Spectrometry

CIL has produced an extensive array of isotope-labeled and unlabeled standards for a wide range of environmental testing areas, including, but not limited to:

- Groundwater, wastewater and drinking water testing standards
- Soil and sediment testing standards
- Ambient air and exhaust gas testing standards
- Food and feed testing standards
- Proficiency testing reference materials
- Ecotoxicology and exposure analysis standards
- US EPA, European Norm and Japanese JIS Methods standards
- Pesticide analysis standards
- Materials testing standards
- Pharmaceutical standards

In terms of product types, CIL Environmental Contaminant Standards range from "legacy" pollutants such as Dioxin/Furans, PCBs, and Organochlorine (OC) Pesticides to emerging contaminants such as Pyrethroid Pesticides, Phosphorous Flame Retardants, and Pharmaceutical and Personal Care Products. While CIL's Environmental Contaminant Standards heritage is deeply rooted in standards for use in GC/MS analyses, LC/MS has become an essential tool in the Environmental analytical lab, and CIL has produced numerous standards designed for use in conjunction with LC/MS analyses. A brief summary of chemical classes and analytical method types follows:

Isotope-Labeled Chlorodioxin Standards
Unlabeled Chlorodioxin Standards
Isotope-Labeled Chlorofuran Standards
Unlabeled Chlorofuran Standards
Isotope-Labeled Bromodioxin Standards
Unlabeled Bromodioxin Standard
Isotope-Labeled Bromofuran Standards
Unlabeled Bromofuran Standards
Isotope-Labeled Mixed Bromo/Chlorodioxin Standards
Unlabeled Mixed Bromo/Chlorodioxin Standards
Unlabeled Mixed Bromo/Chlorofuran Standards
US EPA Method 1613 Standard Mixtures
US EPA Method 23 Standard Mixtures
US EPA Method 8290 Standard Mixtures
US EPA Method 8280 Standard Mixtures
JIS Methods K0311 and K0312 Dioxin/Furan Standard Mixtures
European Air Method EN-1948 Standard Mixtures
Performance Evaluation Reference Materials
Dioxin and Furan plus PCB Standard Mixtures
Non-2,3,7,8-Containing Standard Mixtures
Two Column Dioxin and Furan Standard Mixtures
Mono-Tri Dioxin and Furan Standard Mixtures
Isotope-Labeled Dioxin and Furan Standard Mixtures
Unlabeled Dioxin and Furan Standard Mixtures
Chlorodioxin and Chlorofuran Window Defining Mixtures
TCDD and TCDF Column Performance Mixtures
Bromodioxin/Furan Standard Mixtures
Isotope-Labeled Individual PCB Standards
Unlabeled "Certified" Individual PCB Standards
Unlabeled PCB Standards
US EPA Method 1668A/B Standard Mixtures
CEN Method EN-1948-4 WHO PCB Standard Mixtures
CEN Method EN-1948-4 Marker PCB Standard Mixtures
JIS PCB Methods Standard Mixtures
WHO "Dioxin-Like" PCB Mixtures
Dioxin-Like PCB RH12 Standard Mixtures
WHO "Non-Dioxin-Like" Marker PCB Standard Mixtures
Rapid PCB Screening Standard Mixtures
Mono-Deca plus Predominant PCB Standard Mixtures
Toxic and Predominant PCB Standard Mixtures
CDC PCB Standard Mixtures
Isotope-Labeled PCB Standard Mixtures
Unlabeled PCB Standard Mixtures
PCB Window Defining Mixtures
Isotope-Labeled Mixed Bromo/Chloro Biphenyl Standards
Unlabeled Mixed Bromo/Chloro Biphenyl Standards
Mixed Bromo/Chloro Biphenyl Standard Mixtures
Unlabeled Methyl Sulfone PCB Standards
PCB Metabolite Standards
Isotope-Labeled Individual Brominated Diphenyl Ether (BDE) Standards
Unlabeled Individual Brominated Diphenyl Ether (BDE) Standards
Isotope-Labeled Individual Polybrominated Biphenyl (PBB) Standards
Unlabeled Individual Polybrominated Biphenyl (PBB) Standards
Unlabeled Individual Brominated Diphenyl Ether (BDE) Standards
BDE Metabolite Standards
Tetrabromobisphenol A (TBBPA) and Hexabromocyclododecane (HBCD) Standards
Other Flame-Retardant Standards
BDE Technical Mixtures
US EPA Method 1614 Standard Mixtures
RoHS BDE Standard Mixtures
Brominated Diphenyl Ether (BDE) Standard Mixtures
Brominated Flame Retardant (BFR) Standard Mixtures
^{13}C-Labeled Polycyclic Aromatic Hydrocarbon (PAH) Standards
Deuterium Labeled Polycyclic Aromatic Hydrocarbon (PAH) Standards
Unlabeled Polycyclic Aromatic Hydrocarbon (PAH) Standards
Isotope-Labeled PAH Standard Mixtures
Isotope-Labeled Polychlorinated Naphthalene (PCN) Standards

Unlabeled Polychlorinated Naphthalene (PCN) Standards
Polychlorinated Naphthalene (PCN) Standard Mixtures
Halowax Technical Mixtures
Substituted Benzothiophenes
Chlorobenzene and Chlorophenol Standard Mixtures
US EPA Method 1653A Standard Mixtures
US EPA Method 1653 Standard Mixtures
US EPA CLP DMC Standard Mixtures
US EPA Methods 1624/1625 Standard Mixtures
Personal Care Product Standards
Sex and Steroidal Hormone Standards
Prescription and Non-Prescription Drug Standards
Veterinary and Human Antibiotic Standards
Food and Drinking Water Analysis Standards
Phthalate and Phthalate Metabolite Standards
Nonylphenol, Nonylphenol Ethoxylate and Nonylphenol Carboxylate Standards
Perfluorinated Compound Standards
Nitrosamine Standards
Tobacco Metabolite and Flavoring Standards
Halogenated and Substituted Benzene and Phenol Standards
Endocrine Disrupting Compounds and Xenoestrogen Standards
Chlorinated Diphenyl Ether Standards
Other Industrial Chemical Standards
Explosives Standards
Individual *n*-Alkane Standards
Priority Pollutant Standards
Chlorinated Cyclodiene Pesticide Standards
Organochlorine (OC) Pesticide and Metabolite Standards
Organophosphate (OP) Pesticide and Metabolite Standards
Carbamate Pesticide and Metabolite Standards
Pyrethroid Pesticide and Metabolite Standards
Triazine Herbicide and Metabolite Standards
Toxaphene Standards
Individual Pesticide and Pesticide Metabolite Standards
Pesticide Standard Mixtures
Toxaphene Standard Mixtures
Pesticide Standard Mixtures
Chemical Weapon Metabolite Standards

"In our early phthalate work at the CDC, Cambridge Isotope Labs provided custom ^{13}C standards quickly and accurately. This allowed the scientists in my lab to do gound-breaking work on human exposures to phthalates that continues today. I thank the Environmental Contaminant Standards new product development team and those great synthetic chemists at CIL."

Professor John W. Brock, PhD

Human Biomonitoring: Attogram Level Sensitivity and Consequences for Analytical Standards Purity

Donald G. Patterson Jr., PhD
President, EnviroSolutions Consulting, Inc., Auburn, GA 30011 USA donpatt@etcmail.com

Internal Versus External Dose in Human Exposure Assessment

The objectives of human exposure assessment to environmental chemicals are to quantify the magnitude, duration, frequency and routes of exposure; and to characterize and enumerate the exposed population. There are several ways to do human exposure assessment. The first is the external dose measurement process followed by modeling to predict the individual internal dose. This method usually involves the collection of questionnaire data and a measurement or estimation of concentrations of the chemical(s) in various environmental media such as air, water, soil, dust, food, consumer products, *etc*. This is followed by assumptions of media contact or intake routes that yield a level of applied dose. Predicting levels of toxicants in people using environmental media monitoring is very difficult and involves many assumptions such as: individual lung, intestine and skin absorption coefficients; genetic factors; personal habits; lifestyle factors; nutritional status; and many others.

A second approach to human exposure assessment is the biomonitoring approach which provides exposure estimates that are more directly related to concentrations of the active agent(s) at the target site or organ. Biomonitoring is an assessment of the internal dose by measuring a toxicant (or its metabolite or protein adduct) in human blood, urine, milk, saliva, adipose tissue, or other tissues. The biomonitoring approach provides a direct measure of exposure that integrates exposures from multiple pathways and sources. This approach decreases the uncertainty inherent in exposure assessment by the external dose method and provides a more biologically relevant measure of true exposure. Instead of predicting levels in people, this approach measures levels of toxicants in people and markedly decreases uncertainty in assessing human risk (Sexton *et al.* 2004). An example of the usefulness of the internal dose measurement versus the external dose process is shown in Figure 1.

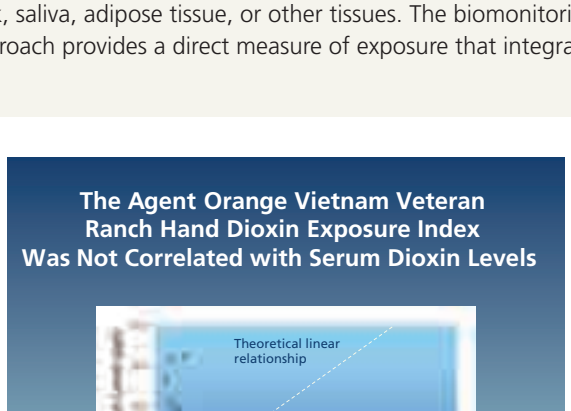


Figure 1.

The US Air Force conducted a 20-year prospective study examining the health, mortality and reproductive outcomes in US Air Force veterans of Operation Ranch Hand (RH), the unit responsible for the aerial spraying of herbicides, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-contaminated Agent Orange, in Vietnam from 1962 to 1971 (Pavuk *et al.* 2007). Prior to beginning the study, the Air Force measured the levels of 2,3,7,8-TCDD in the serum (Patterson *et al.* 1987) of RH veterans and compared the levels to the external dose exposure index that had been developed for the Health Study. Figure 1 shows that the exposure index was poorly correlated with the internal dose TCDD measurements. Based on these results, the Air Force decided to use the internal dose TCDD serum measurements as the exposure index for the Health Study (Michalek 1989).

National Report on Human Exposure to Environmental Chemicals

Before what is "abnormal" may be determined, what is "normal" must be defined. The National Report on Human Exposure to Environmental Chemicals is an ongoing (every two years) biomonitoring assessment of the exposure of the US population to selected environmental chemicals, which are measured in urine, blood and its components. The goals of the National Report are to:

1) Assess exposure to various chemicals
2) Establish national "reference ranges" of these chemicals
3) Track, over time, trends in these "reference ranges"
4) Help set priorities on linking exposure to health outcomes in the American population and subpopulations by age, sex and race/ethnicity.

The samples for the National Report are obtained from the National Health and Nutrition Examination Survey (NHANES), which is conducted by the National Center for Health Statistics

of the Centers for Disease Control and Prevention (CDC). The objective of this survey is to assess the health and nutritional status of adults and children in the United States. The NHANES sampling plan is a complex, stratified, multistage, probability cluster design that selects a representative sample of the civilian, non-institutionalized US population. The data collection includes information from questionnaires, physical examinations on individual participants, chemical measurements and clinical tests on samples collected from about 5,000 participants annually.

Since 1999, NHANES has incorporated a continuous annual survey of persistent organic pollutants (POPs), as well as other chemical measurements that are reported every two years from a random one-third subset of the collected samples. The reference range levels for a number of POPs, including various congeners of the polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (PCBs) and organochlorine pesticides have recently been published for the NHANES 2001-2002 study (Patterson *et al.* 2008) and the NHANES 2003-2004 study (Patterson *et al.* 2009). These results have been reported for the total US population (age 20+) and by age groups (ages 12-19, 20-39, 40-59 and 60+), sex, and race/ethnicity [Mexican American (MA), non-Hispanic blacks (NHB), and non-Hispanic whites (NHW)].

In addition to reporting the reference ranges for the individual congeners, Patterson *et al.* have also reported the total toxic equivalents (TEQ) reference ranges for the US population. Each of the individual PCDD, PCDF and PCB congeners has been assigned a toxic equivalency factor (TEF) relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) by the World Health Organization (WHO). These TEF values (Van den Berg *et al.* 2006) are multiplied by the respective congener concentration to give the congener WHO toxic equivalency (TEQ), and these are summed together to give the total TEQ for each person. In addition, results from the NHANES 2003-2004 survey have been reported for polybrominated diphenyl ethers (Sjodin *et al.* 2008), polycyclic aromatic hydrocarbon metabolites (Li *et al.* 2008) and polyfluoroalkyl chemicals (Calafat *et al.* 2007). Additional classes of chemicals from the latest National Report on Human Exposure to Environmental Chemicals are listed in Figure 2.

Analytical Method Considerations and New Extremely Low Detection Limits

When we began our work on measuring dioxin in human tissues, we used adipose tissue because the levels were higher in this lipid-rich tissue (Patterson *et al.* 1986a).

Figure 2.

Because of the invasive nature of the surgical procedure required to obtain the adipose tissue sample, we had a lower-than-expected participation rate for our first adipose tissue study in Times Beach, Missouri (Patterson *et al.* 1986b). We then turned our attention to developing a method using serum (Patterson *et al.* 1987) which was a less invasive matrix but the levels were much lower in serum due to the small amount of lipid (~0.6%) compared to adipose tissue (~95%). The methods required high-resolution mass spectrometry (HRMS) in order to have the sensitivity required to measure normal background dioxin levels in the picogram to femtogram range. For human studies, we needed the highest accuracy possible which required the use of isotopically labeled internal standards for our quantification scheme.

At the time we began our work, very few unlabeled and isotopically labeled dioxins, furans and PCBs were commercially available. We therefore constructed at the Division of Laboratory Sciences at CDC a special Chemical Toxicant Laboratory (CTL) (Myers and Patterson 1987) and synthesized unlabeled and ^{13}C-labeled PCDD, PCDF and PCB congeners (Figure 3).

The utility of using isotope-dilution quantification is apparent in Figure 4. The 2,3,4,7,8-PeCDF congener (Figure 4a) had a $^{13}C_{12}$-2,3,4,7,8-PeCDF congener as an internal standard and the accuracy of the measured concentration versus the expected concentration is apparent.

Figure 4b shows the quantitative results for the 1,2,3,4,7,8,9-HpCDF congener which did not have a ^{13}C-labeled internal standard. The inaccuracy for this congener is apparent in Figure 4b. Over the years, unlabeled and isotopically labeled standards became available from

(continued)

Figure 3.

Cambridge Isotopes Laboratories, Inc. (CIL) Many of the analytes measured by CDC in the NHANES surveys described above use CIL unlabeled and ^{13}C-labeled standards. The CIL unlabeled standards provide the accuracy base for all these analytes in the NHANES studies which provide background national reference ranges for these chemicals in people from the United States.

For a number of reasons, it is important to continue to try to develop more sensitive analytical methods for environmental chemicals:

1) to determine the normal human background levels of chemicals shown to be toxic to certain animals that we cannot detect with current methods;
2) to continue monitoring chemical levels that are decreasing in the US population (dioxins, furans, PCBs, pesticides);
3) to provide better analytical CVs of chemicals that we can measure which will translate into lower measurement uncertainties; and
4) a lower analytical CV translates directly into higher statistical power in epidemiological studies.

A lower analytical CV allows a higher statistical power for a given number of samples in an epidemiological study. Also, a lower analytical CV can provide the same statistical power using a smaller numbers of samples in a study (generating a cost savings).

Newer, more sensitive analytical techniques are currently being developed (Patterson *et al.* 2011) using cryogenic zone compression and loop modulation coupled with high resolution mass spectrometry to measure persistent organic pollutants. A chromatogram showing the signal from a standard of 2,3,7,8-TCDD (313 attogram) using this newer technique is depicted in Figure 5.

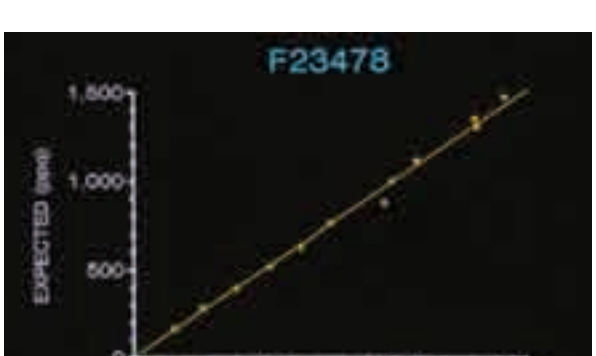


Figure 4a.

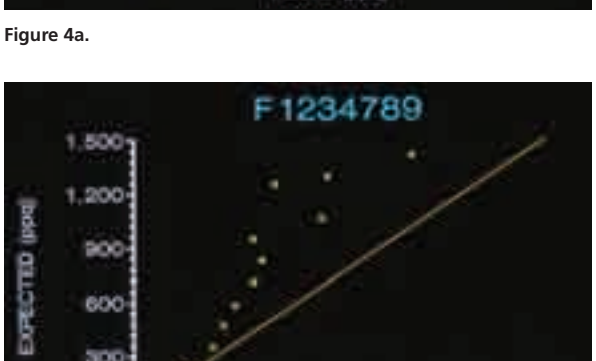


Figure 4b.

A modification of this technique, called time-controlled cryogenic zone compression, being developed by Thermo Scientific, is shown in Figure 6.

This technique allows targeted cryofocusing of certain peaks that might need enhanced sensitivity while allowing the remainder of the chromatographic separation to proceed unaltered. Tables 1 and 2 summarize the current state of the art in sensitivity for measurements of dioxin and dioxin-like chemicals.

Table 1

Sensitivity for 2,3,7,8-TCDD using various GC–MS techniques

Technique	Sample amount on column	S/N (4σ)
GC (MAT95XP)–HRMS	Standard 20 fg	43
GC (DFS)–HRMS	Standard 20 fg	604
CZC-GC (MAT95XP)–HRMS	Standard 313 ag	400
CZC-GC (MAT95XP)–HRMS	Serum 325 ag	161
GCxGC-LRTOFMS	Standard 500 fg	6

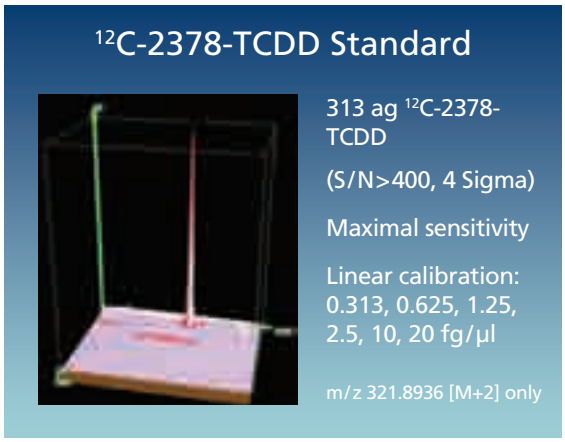


Figure 5.

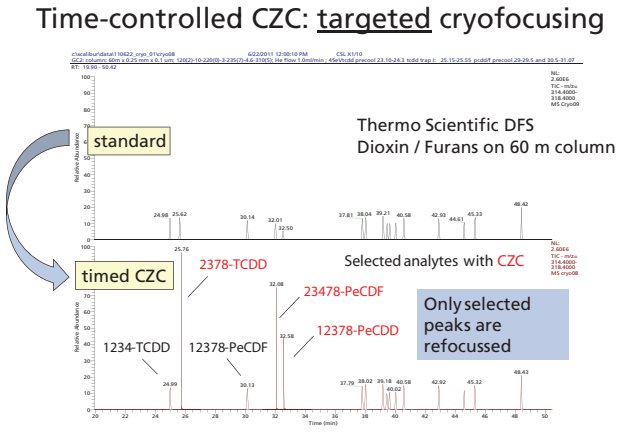


Figure 6.

Table 2			
Current state of the art for the measurement of 2,3,7,8-TCDD and the potential detection limits and numbers of molecules (calculations based on M+2 321.8936 m/z ion).			
Quantity	Notation	Number of moles	Number of molecules
1 nanogram (ng) 10^{-9} g	ppb	3.1×10^{-12} (3.1 picomoles)	1,870,000,000,000 (1.87×10^{12})
1 picogram (pg) 10^{-12} g	ppt	3.1×10^{-15} (3.1 femtomoles)	1,870,000,000 (1.87×10^{9})
1 femtogram (fg) 10^{-15} g	ppq	3.1×10^{-18} (3.1 attomoles)	1,870,000 (1.87×10^{6})
313 attogram (ag) 10^{-18} g	**ppquint**	**9.7×10^{-19} (972 zeptomoles)**	**586,000 (5.86×10^{5})**

The consequences of the use of these newer analytical techniques for CIL and other laboratories producing and supplying analytical standards is that the purity of the standards will most likely have to be improved. Even very small amounts of the unlabeled compound or partially labeled compound in isotopically labeled standards will be detectable and interfere with accurate quantification. For example, in 1 ng of a standard, 0.00001% impurity is 100 attograms! Impurities at these levels will be detectable and will have to be eliminated. This could be a time-consuming and costly process for standard producers which could require extensive laboratory facility cleanup and extensive quality assurance/quality control procedures.

References

Calafat, A.M.; Wong, L-Y.; Kuklenyik, Z.; Reidy, J.A.; and Needham, L.L. **2007**. *Environ Health Perspect, 115,* 1596-1602.

Li, Z.; Sandau, C.D.; Romanoff, L.C.; Caudill, S.P. Sjodin, A.; Needham, L.L.; Patterson Jr., D.G. **2008**. *Environ Res, 103,* 320-331.

Michalek, J.E. **1989**. *Applied Industrial Hygiene, 12/89,* 68-72.

Myers, G.L.; and Patterson, D.G. **1987**. *Professional Safety, 32,* 30-37.

Patterson Jr., D.G.; Holler, J.S.; Lapeza Jr., C.R.; Alexander, L.R.; Groce, D.F.; O'Connor, R.C.; Smith, S.J.; Liddle, J.A.; Needham, L.L. **1986a**. *Anal Chem, 58,* 705-713.

Patterson Jr., D.G.; Hoffman, R.E.; Needham, L.L.; Roberts, D.W.; Bagby, J.R.; Pirkle, J.L.; Falk, H.; Sampson, E.J.; Houk, V.N. **1986b**. *JAMA, 256,* 2683-2686.

Patterson Jr., D.G.; Hampton, L.; Lapeza Jr., C.R.; Belser, W.T.; Green, V.; Alexander, L.; Needham, L.L. **1987**. *Anal Chem, 59,* 2000-2005.

Patterson Jr., D.G.; Turner, W.E.; Caudill, S.P.; Needham, L.L. **2008**. *Chemosphere, 73,* S261-S277.

Patterson Jr., D.G.; Wong, L-Y.; Turner, W.E.; Caudill, S.P.; Dipietro, E.S.; McClure, P.C.; Cash, T.P.; Osterloh, J.D.; Pirkle, J.L.; Sampson, E.J.; Needham, L.L. **2009**. *Environ Sci Technol, 43,* 1211-1218.

Patterson Jr., D.G.; Welch, S.M.; Turner, W.E.; Sjödin, A.; Focant, J-F. **2011**. *J Chromatography A, 1218,* 3274-3281.

Pavuk, M.; Patterson Jr., D.G.; Turner, W.E.; Needham, L.L.; Ketchum, N.S. **2007**. *Chemosphere, 68,* 62-68.

Sexton, K.; Needham, L.L.; and Pirkle, J.L. **2004**. *American Scientist, 92,* 38-45.

Sjodin, A.; Wong, L-Y.; Jones, R.S.; Park, A.; Zhang, Y.; Hodge, C.; Dipietro, E.; McClure, C.; Turner, W.; Needham, L.L.; and Patterson Jr., D.G. **2008**. *Environ Sci Technol, 42,* 1377-1384.

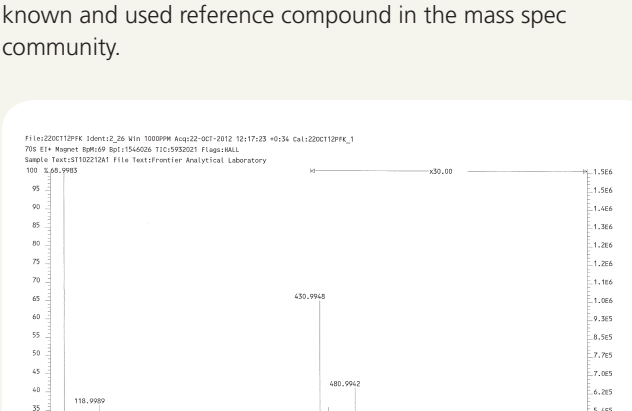
Van den Berg, M.; Birnbaum, L.; Denison, M.; DeVito, M.; Farland, W.; Feeley, M.; Fiedler, H.; Hakansson, H.; Hanberg, A.; Haws, L.; Rose, M.; Safe, S.; Schrenk, D.; Tohyama, C.; Tritscher, A.; Tuomisto, J.; Tysklind, M.; Walker, N.; Peterson, R.E. **2006**. *Toxicol Sci, 93,* 223–241.

Perfluorokerosene: A Historical Perspective

Tom Crabtree, Brad Silverbush, Dan Vickers
Frontier Analytical Laboratory
El Dorado Hills, CA 95762 USA

Mass spectrometers require a reference compound to accurately assign masses and to verify tuning and operating condition of the instrument. This compound is typically introduced into the mass spectrometer via a separate inlet, and continually bled into the instrument during analysis. After tuning and calibration, the instrument's data system continually monitors selected fragments of the reference compound to compensate for mass drift over time. These reference compounds are specially formulated chemicals designed to be easily recognized by their unique fragmentation patterns. Ideally, such a reference material does not contaminate, obfuscate or interact with the analytes of interest.

In the late 1960s, Columbia Organic Chemical Company successfully synthesized perfluorokerosene (PFK) and published a negative ion EI spectra in the Journal of Analytical Chemistry, 1968, Volume 40, Issue 6, pg.1004-1006. Researchers immediately recognized the usefulness of PFK while performing EI analysis, and subsequently PFK became the most widely known and used reference compound in the mass spec community.



On July 9, 1970, President Richard M. Nixon signed an executive order establishing the US Environmental Protection Agency (US EPA) for the purpose of protecting human health and the environment. It had become clear that past industrial neglect and resulting environmental contaminations were having a negative impact on the health of the population. Huge tracts of land, groundwater, lakes and waterways were found to be contaminated with near toxic levels of dioxins and PCBs. Some chemical processes unintentionally produce dioxin as a byproduct. This became clear when a chemical defoliant used during the Vietnam War known as "Agent Orange" was found to contain high levels of dioxin. Incomplete combustion of certain types of plastics and chlorinated chemicals can also produce dioxin. An entire industry grew around identifying and dealing with these environmental contaminants. The US EPA developed many Environmental Contaminant Methodologies for the analysis of dioxins and PCBs (EPA Methods 23, 428, 613, 1613, 1668, 8280, 8290, *etc.*) citing PFK as the suggested reference compound to be used for these analyses.

Owing to the difficulty of synthesizing and purifying PFK with desirable properties such as specific boiling point range, as of 2011 there was only one specialty chemical company producing PFK. This company has since halted production of PFK. The last of the High Boiling PFK in the chemical inventory was sold sometime early in 2012

Cambridge Isotope Laboratories, Inc. recognized the need for continued production of PFK in environmental analytical laboratories and has partnered with a new producer, with clean material tested by Frontier Analytical. Low Boiling PFK and High Boiling PFK versions are available in gram and sub-gram sizes.

Related Products

Catalog No.	Description
PFK-HIGH-0.1	Perfluorokerosene, high-boiling range (unlabeled) 0.1 g
PFK-HIGH-0.5	Perfluorokerosene, high-boiling range (unlabeled) 0.5 g
PFK-HIGH-1	Perfluorokerosene, high-boiling range (unlabeled) 1 g
PFK-LOW-0.25	Perfluorokerosene, low-boiling range (unlabeled) 0.25 g
PFK-LOW-1	Perfluorokerosene, low-boiling range (unlabeled) 1 g

Phthalate and Phthalate Metabolite Standards

Catalog No.	Description
DLM-1369-1.2	Benzyl butyl phthalate (ring-D_4, 98%) 100 µg/mL in nonane
ULM-7551-1.2	Benzyl butyl phthalate (unlabeled) 100 µg/mL in nonane
CLM-4675-1.2	Bis(2-ethylhexyl) adipate (adipate-$^{13}C_6$, 99%) 100 µg/mL in nonane
ULM-6566-1.2	Bis(2-ethylhexyl) adipate (unlabeled) 100 µg/mL in nonane
DLM-1368-1.2	Bis(2-ethylhexyl) phthalate (ring-D_4, 98%) 100 µg/mL in nonane
ULM-6241-1.2	Bis(2-ethylhexyl) phthalate (unlabeled) 1000 µg/mL in nonane
DLM-1367-1.2	Di-*n*-butyl phthalate (ring-D_4, 98%) 100 µg/mL in nonane
ULM-7466-1.5	Di-*n*-butyl phthalate (unlabeled) 100 µg/mL in nonane
CLM-4670-1.2	Dicyclohexyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in nonane
ULM-8785-1.2	Dicyclohexyl phthalate (unlabeled) 100 µg/mL in nonane
DLM-1629-1.2	Diethyl phthalate (ring-D_4, 98%) 100 µg/mL in nonane
ULM-6174-1.2	Diethyl phthalate (unlabeled) 100 µg/mL in nonane
CLM-4669-1.2	Di-*n*-hexyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in nonane
ULM-7434-1.2	Di-*n*-hexyl phthalate (unlabeled) 100 µg/mL in nonane
DLM-1366-1.2	Dimethyl phthalate (ring-D_4, 98%) 100 µg/mL in nonane
ULM-6783-1.2	Dimethyl phthalate (unlabeled) 100 µg/mL in nonane
DLM-1630-1.2	Di-*n*-octyl phthalate (ring-D_4, 98%) 100 µg/mL in nonane
ULM-6129-1.2	Di-*n*-octyl phthalate (unlabeled) 100 µg/mL in nonane
CLM-4668-1.2	Di-*n*-pentyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in nonane
ULM-7433-1.2	Di-*n*-pentyl phthalate (unlabeled) 100 µg/mL in nonane
CLM-4591-MT-1.2	Monobenzyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-6149-MT-1.2	Monobenzyl phthalate (unlabeled) 100 µg/mL in MTBE
CLM-6148-MT-1.2	Mono-*n*-butyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-6148-MT-1.2	Mono-*n*-butyl phthalate (unlabeled) 100 µg/mL in MTBE
CLM-8148-MT-1.2	Mono-(2-ethyl-5-carboxy-pentyl) phthalate (DEHP Metabolite V) ($^{13}C_4$, 99%) 100 µg/mL in MTBE
ULM-8149-MT-1.2	Mono-(2-ethyl-5-carboxy-pentyl) phthalate (DEHP Metabolite V) (unlabeled) 100 µg/mL in MTBE
CLM-8232-MT-1.2	Mono-[(2-carboxymethyl) hexyl] phthalate (DEHP Metabolite IV) ($^{13}C_4$, 99%) 100 µg/mL in MTBE
ULM-8233-MT-1.2	Mono-[(2-carboxymethyl) hexyl] phthalate (DEHP Metabolite IV) (unlabeled) 100 µg/mL in MTBE

Catalog No.	Description
CLM-6847-MT-1.2	Mono-(3-carboxypropyl) phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-6848-MT-1.2	Mono-(3-carboxypropyl) phthalate (unlabeled) 100 µg/mL in MTBE
CLM-4592-MT-1.2	Monocyclohexyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-7394-MT-1.2	Monocyclohexyl phthalate (unlabeled) 100 µg/mL in MTBE
CLM-4584-MT-1.2	Mono-2-ethylhexyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-4583-MT-1.2	Mono-2-ethylhexyl phthalate (unlabeled) 100 µg/mL in MTBE
CLM-6641-MT-1.2	Mono-(2-ethyl-5-hydroxyhexyl) phthalate (DEHP Metabolite IX) (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C^2$, 99%) 100 µg/mL in MTBE
ULM-4662-MT-1.2	Mono-(2-ethyl-5-hydroxyhexyl) phthalate (DEHP Metabolite IX) (unlabeled) 100 µg/mL in MTBE
CLM-6640-MT-1.2	Mono-(2-ethyl-5-oxohexyl) phthalate (DEHP Metabolite VI) ($^{13}C_4$, 99%) 100 µg/mL in MTBE
ULM-4663-MT-1.2	Mono-(2-ethyl-5-oxohexyl) phthalate (DEHP Metabolite VI) (unlabeled) 100 µg/mL in MTBE
CLM-4586-MT-1.2	Monoethyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-4585-MT-1.2	Monoethyl phthalate (unlabeled) 100 µg/mL in MTBE
ULM-7919-MT-1.2	Monoisobutyl phthalate (unlabeled) 100 µg/mL in MTBE
ULM-4652-1.2	Monoisodecyl phthalate (Mono-3,7-dimethyloctyl phthalate) (unlabeled) 100 µg/mL in acetonitrile
CLM-4587-MT-1.2	Monoisononyl phthalate (Mono-3,5,5-trimethylhexyl phthalate) (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-4651-MT-1.2	Monoisononyl phthalate (Mono-3,5,5-trimethylhexyl phthalate) (unlabeled) 100 µg/mL in MTBE
ULM-7395-1.2	Monoisopropyl phthalate (unlabeled) 100 µg/mL in acetonitrile
CLM-6071-1.2	Monomethyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in acetonitrile
ULM-6697-MT-1.2	Monomethyl phthalate (unlabeled) 100 µg/mL in MTBE
CLM-4589-MT-1.2	Mono-*n*-octyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-4593-MT-1.2	Mono-*n*-octyl phthalate (unlabeled) 100 µg/mL in MTBE
ULM-7393-1.2	Mono-*n*-pentyl phthalate (unlabeled) 100 µg/mL in acetonitrile

See pages 152-153 for a list of Pharmaceutical and Personal Care Products (PPCPs).

Prescription and Non-Prescription Drug Standards

Catalog No.	Description
CNLM-3726-1.2	Acetaminophen (acetyl-$^{13}C_2$, 99%;^{15}N, 98%) 100 µg/mL in acetonitrile
ULM-7629-1.2	Acetaminophen (unlabeled) 100 µg/mL in acetonitrile
DLM-3008-1.2	Amitriptyline•HCl (*N,N*-dimethyl-D_6, 98%) 100 µg/mL in methanol
ULM-8350-1.2	Amitriptyline•HCl (unlabeled) 100 µg/mL in methanol
CLM-514-1.2	Caffeine (trimethyl-$^{13}C_3$, 99%) 100 µg/mL in methanol
ULM-7653-1.2	Caffeine (unlabeled) 100 µg/mL in methanol
DLM-2806-1.2	Carbamazepine (D_{10}, 98%) 100 µg/mL in acetonitrile-D_3
ULM-6581-1.2	Carbamazepine (unlabeled) CP 97% 100 µg/mL in acetonitrile
DLM-1287-1.2	Clonidine (4,4,5,5-imidazoline-D_4, 98%) 100 µg/mL in methanol
ULM-8349-1.2	Clonidine (unlabeled) 100 µg/mL in methanol
C-041	Codeine (D_6, 98%) 1.0 mg/mL in methanol
C-006	Codeine (unlabeled) 1.0 mg/mL in methanol
C-035	(+/-)-Cotinine (D_3, 98%) 1.0 mg/mL in methanol
C-016	(-)-Cotinine (unlabeled) 1.0 mg/mL in methanol
D-902	Diazepam (D_5, 98%) 100 µg/mL in methanol
D-907	Diazepam (unlabeled) 1.0 mg/mL in methanol
DLM-8567-1.2	Diclofenac (phenyl-D_4, 98%) 100 µg/mL in methylene chloride
ULM-9023-1.2	Diclofenac (unlabeled) 100 µg/mL in methylene chloride
CNLM-411-1.2	5,5-Diphenylhydantoin (2-^{13}C, 99%;1,3-$^{15}N_2$, 98%) 100 µg/mL in methanol
ULM-8533-1.2	5,5-Diphenylhydantoin (unlabeled) 100 µg/mL in methanol
F-919	Fluoxetine oxalate (D_6, 98%) 100 µg/mL in methanol
F-918	Fluoxetine•HCl (unlabeled) 1.0 mg/mL in methanol
DLM-8221-1.2	Gemfibrozil (2,2-dimethyl-D_6, 98%) 100 µg/mL in *p*-dioxane
ULM-8225-1.2	Gemfibrozil (unlabeled) 100 µg/mL in *p*-dioxane
CLM-6943-1.2	Ibuprofen (propionic-$^{13}C_3$, 99%) 100 µg/mL in acetonitrile
ULM-7275-1.2	Ibuprofen (unlabeled) 100 µg/mL in acetonitrile
I-902	Imipramine (unlabeled) 1.0 mg/mL in methanol
L-902	Lorazepam (D_4, 98%) 100 µg/mL in acetonitrile
L-901	Lorazepam (unlabeled) 1.0 mg/mL in acetonitrile
CDLM-7665-1.2	Naproxen (methyl-^{13}C, 99% methyl-D_3, 98%) 100 µg/mL in acetonitrile
ULM-7709-1.2	Naproxen (unlabeled) 100 µg/mL in acetonitrile
N-922	Norfluoxetine oxalate (D_6, 98%) 100 µg/mL in methanol
N-923	Norfluoxetine oxalate (unlabeled) 1.0 mg/mL in methanol
DLM-3039-1MG	Phenylbutazone (diphenyl-D_{10}, 98%) neat
ULM-7378-1MG	Phenylbutazone (unlabeled) neat
CLM-7892	Resorcinol ($^{13}C_6$, 99%)
CLM-8370-1.2	Thiabendazole (ring-$^{13}C_6$, 99%) 100 µg/mL in acetonitrile
ULM-8371-1.2	Thiabendazole (unlabeled) 100 µg/mL in acetonitrile
DLM-6861-1.2	Warfarin (phenyl-D_5, 98%) 100 µg/mL in acetonitrile-D_3
ULM-7242-1.2	Warfarin (unlabeled) 100 µg/mL in acetonitrile

Veterinary and Human Antibiotic Standards

Catalog No.	Description
DLM-7170-1.2	1-Aminohydantoin hydrochloride (AHD) (5,5-D_2, 98%) 100 µg/mL in acetonitrile-D_3
ULM-7188-1.2	1-Aminohydantoin hydrochloride (AHD) (unlabeled) 100 µg/mL in methanol
DLM-7171-1.2	3-Amino-2-oxazolidone (AOZ) (ring-D_4, 98%) 100 µg/mL in acetonitrile-D_3
ULM-7189-1.2	3-Amino-2-oxazolidone (AOZ) (unlabeled) 100 µg/mL in methanol
CLM-7407-1MG	Amoxicillin•3H_2O (phenyl-$^{13}C_6$, 99%) neat
DLM-119-1.2	(+/-)-Chloramphenicol (ring-D_4, benzyl-D_1, 98%) 100 µg/mL in acetonitrile
ULM-6687-1.2	(+/-)-Chloramphenicol (unlabeled) 100 µg/mL in acetonitrile
CNLM-7539-1.2	Ciprofloxacin•HCl (2,3,carboxyl-$^{13}C_3$, 99%; quinoline-^{15}N, 98%) 100 µg/mL in methanol
ULM-7710-1.2	Ciprofloxacin•HCl (unlabeled) 100 µg/mL in methanol
CLM-3672-1.2	Erythromycin (90-95% Erythromycin A) (*N,N*-dimethyl-$^{13}C_2$, ~90%) 100 µg/mL in acetonitrile
ULM-4322-1.2	Erythromycin (unlabeled) 100 µg/mL in acetonitrile
DLM-7172-1.2	5-(4-Morpholinylmethyl)-3-amino-2-oxazolidinone (AMOZ) (4,4,5,5′,5′-D_5, 98%) 100 µg/mL in acetonitrile-D_3
ULM-7190-1.2	5-(4-Morpholinylmethyl)-3-amino-2-oxazolidinone (AMOZ) (unlabeled) 100 µg/mL in methanol
CLM-3045-1.2	Sulfamethazine (phenyl-$^{13}C_6$, 90%) 100 µg/mL in acetonitrile
ULM-7220-1.2	Sulfamethazine (unlabeled) 100 µg/mL in acetonitrile
CLM-6944-1.2	Sulfamethoxazole (ring-$^{13}C_6$, 99%) 100 µg/mL in acetonitrile
ULM-7527-1.2	Sulfamethoxazole (unlabeled) 100 µg/mL in acetonitrile
CLM-7988-A-1.2	Trimethoprim (pyrimidine-4,5,6-$^{13}C_3$, 99%) 50 µg/mL in methanol
ULM-7989-A-1.2	Trimethoprim (unlabeled) 50 µg/mL in methanol

Steroids

Catalog No.	Description
DLM-8438	Aldosterone (2,2,4,6,6,17,21,21-D_8)
ULM-9134	Aldosterone (unlabeled) CP 95%
DLM-8750	5β-Androstan-3α-ol-17-one (16,16-D_2, 98%)
CLM-9135	4-Androstene-3,17-dione (2,3,4-$^{13}C_3$, 99%)
CLM-9135-C	4-Androstene-3,17-dione (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in 1,2-dimethoxyethane
CLM-9135-D	4-Androstene-3,17-dione (2,3,4-$^{13}C_3$, 99%) 1000 µg/mL in 1,2-dimethoxyethane
DLM-7976	4-Androstene-3,17-dione (2,2,4,6,6,16,16-D_7, 97%)
ULM-8472	4-Androstene-3,17-dione (unlabeled)
ULM-8472-C	4-Androstene-3,17-dione (unlabeled) 100 µg/mL in 1,2-dimethoxyethane
ULM-8472-D	4-Androstene-3,17-dione (unlabeled) 1000 µg/mL in 1,2-dimethoxyethane
DLM-7937	Androsterone (16,16-D_2, 98%)
DLM-9137	Androsterone glucuronide (2,2,4,4-D_4, 98%)
ULM-9138	Androsterone glucuronide (unlabeled)
DLM-6780	Chenodeoxycholic acid (2,2,4,4-D_4, 98%)
DLM-4700	Cholestane (3,3-D_2, 98%)
DLM-8276	Cholestenone (2,2,4,6,6-D_5, 98%)
CLM-9139	Cholesterol (2,3,4-$^{13}C_3$, 99%)
CLM-9139-B	Cholesterol (2,3,4-$^{13}C_3$, 99%) 50 µg/mL in chloroform
CLM-9139-C	Cholesterol (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in chloroform
CLM-804	Cholesterol (3,4-$^{13}C_2$, 99%)
DLM-1831	Cholesterol (3-D_1, 98%)
DLM-7260	Cholesterol (25,26,26,26-D_4, 98%)
DLM-2607	Cholesterol (2,2,3,4,4,6-D_6, 97-98%)
DLM-3057	Cholesterol (25,26,26,26,27,27,27-D_7, 98%)
OLM-7695	Cholesterol (^{18}O, 80%)
ULM-9140	Cholesterol (unlabeled)
ULM-9140-C	Cholesterol (unlabeled) 100 µg/mL in chloroform
ULM-9140-D	Cholesterol (unlabeled) 1000 µg/mL in chloroform
CLM-3361	Cholesterol-3-octanoate (octanoate-1-^{13}C, 99%)
CLM-2710	Cholic acid (24-^{13}C, 99%)
DLM-2611	Cholic acid (2,2,4,4-D_4, 98%)
DLM-7347	Corticosterone (2,2,4,6,6,17a,21,21-D_8, 97-98%)
DLM-2615	Cortisol (1,2-D_2, 98%)
DLM-2057	Cortisol (9,12,12-D_3, 98%)
DLM-2218	Cortisol (9,11,12,12-D_4, 98%)
ULM-9141	Cortisol (unlabeled)
ULM-9141-C	Cortisol (unlabeled) 100 µg/mL in methanol
ULM-9141-D	Cortisol (unlabeled) 1000 µg/mL in methanol
ULM-7823	Cortisol (unlabeled)
DLM-8863	Cortisone (1,2-D_2, 98%)
DLM-9142	Cortisone (2,2,4,6,6,12,12-D_7, 98%)
ULM-9202	Cortisone (unlabeled)
DLM-4216	7-Dehydrocholesterol (25,26,26,26,27,27,27-D_7, 98%)
DLM-7714	Dehydroepiandrosterone (DHEA) (16,16-D_2, 97%)
ULM-9143	Dehydroepiandrosterone (DHEA) (unlabeled)
ULM-9143-C	Dehydroepiandrosterone (DHEA) (unlabeled) 100 µg/mL in methanol
ULM-9143-D	Dehydroepiandrosterone (DHEA) (unlabeled) 1000 µg/mL in methanol
DLM-8701	Dehydroepiandrosterone sulfate•sodium salt (DHEAS) (16,16-D_2, 97%)
DLM-8337	Dehydroepiandrosterone sulfate•sodium salt (DHEAS) (2,2,3,4,4,6-D_6, 98%)
ULM-9144	Dehydroepiandrosterone sulfate•sodium salt (DHEAS) (unlabeled)
ULM-9144-C	Dehydroepiandrosterone sulfate•sodium salt (DHEAS) (unlabeled) 100 µg/mL in methanol
ULM-9144-D	Dehydroepiandrosterone sulfate•sodium salt (DHEAS) (unlabeled) 1000 µg/mL in methanol
CLM-3364	Deoxycholic acid (24-^{13}C, 99%)
DLM-2824	Deoxycholic acid (2,2,4,4-D_4, 98%)
DLM-7209	11-Deoxycortisol (21,21-D_2, 96%)
ULM-9145	11-Deoxycortisol (unlabeled)
ULM-9145-C	11-Deoxycortisol (unlabeled) 100 µg/mL in methanol
ULM-9145-D	11-Deoxycortisol (unlabeled) 1000 µg/mL in methanol
DLM-8305	21-Deoxycortisol (D_8, 96%)
DLM-170	Diethylstilbestrol (*cis*/*trans* mix) (ring-3,3′,5,5′-diethyl-1,1,1′,1′-D_8, 98%)
ULM-7921	Diethylstilbestrol (*cis*/*trans* mix) (unlabeled)
DLM-3023	Dihydrotestosterone (16,16,17-D_3, 98%)
CLM-9146	5α-Dihydrotestosterone (2,3,4-$^{13}C_3$, 99%) CP 97%
CLM-9146-C	5α-Dihydrotestosterone (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in 1,2-dimethoxyethane
CLM-9146-D	5α-Dihydrotestosterone (2,3,4-$^{13}C_3$, 99%) 1000 µg/mL in 1,2-dimethoxyethane
DLM-9041	5α-Dihydrotestosterone (2,2,4,4-D_4, 98%)
CNLM-7889	DL-Epinephrine (1,2-$^{13}C_2$, 99%; ^{15}N, 98%)
DLM-2866	DL-Epinephrine (α,α,β-D_3, 97%)
CLM-7936	Estradiol (13,14,15,16,17,18-$^{13}C_6$, 99%) 100 µg/mL in methanol
DLM-3694	Estradiol (16,16,17-D_3, 98%)
DLM-2487	Estradiol (2,4,16,16-D_4, 95-97%)
ULM-7449	Estradiol (unlabeled) 100 µg/mL in nonane
CLM-9147	Estriol (16α-hydroxyestradiol) (2,3,4-$^{13}C_3$, 99%)
CLM-9147-A	Estriol (16α-hydroxyestradiol) (2,3,4-$^{13}C_3$, 99%) 5 µg/mL in methanol
CLM-9147-B	Estriol (16α-hydroxyestradiol) (2,3,4-$^{13}C_3$, 99%) 50 µg/mL in methanol
CLM-9147-C	Estriol (16α-hydroxyestradiol) (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in methanol
DLM-7468	Estriol (2,4-D_2, 98%)
DLM-8343	Estriol (2,4,17-D_3, 98%) CP 96%
DLM-8583	Estriol (2,4,16,17-D_4, 98%) CP 95%
DLM-8586	Estriol (2,4,16-D_3, 98%)
ULM-8218	Estriol (unlabeled)
CLM-9148	Estrone (2,3,4-$^{13}C_3$, 99%)
CLM-9148-B	Estrone (2,3,4-$^{13}C_3$, 99%) 50 µg/mL in methanol
CLM-9148-C	Estrone (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in methanol
CLM-673	Estrone (3,4-$^{13}C_2$, 99%) 100 µg/mL in acetonitrile
DLM-3976	Estrone (2,4,16,16-D_4, 97%)
CLM-8033	DL-Estrone 3-methyl ether (13,14,15,16,17,18-$^{13}C_6$, 99%)
CLM-3375	Ethynylestradiol (20,21-$^{13}C_2$, 99%) 100 µg/mL in acetonitrile
DLM-4691	17-α-Ethynylestradiol (2,4,16,16-D_4, 97-98%)
ULM-7211	Ethynylestradiol (unlabeled) 100 µg/mL in acetonitrile

(continued)

Steroids

Catalog No.	Description
DLM-8646	7-β-Hydroxycholesterol (25,26,26,26,27,27,27-D_7, 98%) CP 97%
DLM-9150	18-Hydroxycorticosterone (9,11,12,12-D_4, 98%) CP 95%
ULM-9151	18-Hydroxycorticosterone (unlabeled) CP 95%
DLM-9149	6β-Hydroxycortisol (9,11,12,12-D_4) CP 97%
CLM-8012	DL-2-Hydroxyestradiol (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8135	2-Hydroxyestradiol (unlabeled)
ULM-8134	2-Hydroxyestrone (unlabeled)
CLM-8013	DL-4-Hydroxyestrone (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8261	4-Hydroxyestrone (unlabeled) CP 96%
CLM-9153	16α-Hydroxyestrone (2,3,4-$^{13}C_3$, 99%)
ULM-9152	16α-Hydroxyestrone (unlabeled)
CLM-8016	DL-2-Hydroxyestrone-3-methyl ether (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8133	2-Hydroxyestrone-3-methyl ether (unlabeled)
DLM-7206	17-Hydroxypregnenolone (21,21,21-D_3, 97%)
CDLM-9154	17α-Hydroxypregnenolone (20,21-$^{13}C_2$, 99%; 16,16-D_2, 99%)
CDLM-9154-C	17α-Hydroxypregnenolone (20,21-$^{13}C_2$, 99%; 16,16-D_2, 99%) 100 µg/mL in methanol
CDLM-9154-D	17α-Hydroxypregnenolone (20,21-$^{13}C_2$, 99%; 16,16-D_2, 99%) 1000 µg/mL in methanol
ULM-9155	17α-Hydroxypregnenolone (unlabeled)
ULM-9155-C	17α-Hydroxypregnenolone (unlabeled) 100 µg/mL in methanol
ULM-9155-D	17α-Hydroxypregnenolone (unlabeled) 1000 µg/mL in methanol
CLM-9157	17α-Hydroxyprogesterone (2,3,4-$^{13}C_3$, 98%)
CLM-9157-C	17α-Hydroxyprogesterone (2,3,4-$^{13}C_3$, 98%) 100 µg/mL in methanol
CLM-9157-D	17α-Hydroxyprogesterone (2,3,4-$^{13}C_3$, 98%) 1000 µg/mL in methanol
DLM-6598	17-Hydroxyprogesterone (2,2,4,6,6,21,21,21-D_8, 98%)
ULM-9156	17α-Hydroxyprogesterone (unlabeled)
ULM-9156-C	17α-Hydroxyprogesterone (unlabeled) 100 µg/mL in methanol CP 95%
ULM-9156-D	17α-Hydroxyprogesterone (unlabeled) 1000 µg/mL in methanol CP 95%
DLM-8647	7-Ketocholesterol (25,26,26,26,27,27,27-D_7, 99%)
DLM-3560	DL-Metanephrine•HCl (α,β,β-D_3, 98%)
CLM-8015	DL-2-Methoxyestradiol (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8137	2-Methoxyestradiol (unlabeled)
CLM-8014	DL-2-Methoxyestrone (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8263	2-Methoxyestrone (unlabeled)
CLM-8017	DL-4-Methoxyestrone (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8262	4-Methoxyestrone (unlabeled)
CLM-2468	Norethindrone (ethynyl-$^{13}C_2$, 99%)
DLM-3670	DL-Norepinephrine•HCl (1,2,2-D_3, 95%)
DLM-8820	DL-Norepinephrine•HCl (ring-D_3,1,2,2-D_3, 99%)
DLM-3979	19-Nortestosterone (16,16,17-D_3, 98%)
ULM-4841	19-Nortestosterone (unlabeled)
ULM-222	Pregna-1,4,6-triene-3,20-dione (unlabeled)
DLM-3754	5-α-Pregnan-3-α-ol-20-one (17,21,21,21-D_4, 96-98%) CP 95%+
ULM-3779	5-α-Pregnan-3-α-ol-20-one (unlabeled) CP 97%
DLM-7492	5-α-Pregnan-3-β-ol-20-one (17α,21,21,21-D_4, 97%+) CP 96%
ULM-8242	5-α-Pregnan-3-β-ol-20-one (unlabeled)
DLM-2294	5-β-Pregnan-3-α-ol-20-one (17,21,21,21-D_4, 96-98%)

Catalog No.	Description
DLM-8751	5-β-Pregnan-3-α,11-β,17-α,21-tetrol-20-one (9,11α,12-D_3, 95%)
DLM-8753	5-β-Pregnan-3-α,17-α,20-triol (20,21,21,21-D_4, 98%) mix of 20α & 20β
DLM-3910	5-α-Pregnane-3-α,21-diol-20-one (17,21,21-D_3, 95%)
DLM-3816	5-α-Pregnane-3,20-dione (1,2,4,5,6,7-D_6, 95%)
DLM-3817	5-β-Pregnane-3,20-dione (1,2,4,5,6,7-D_6, 95%)
DLM-7228	4-Pregnen-21-ol-3,20-dione (2,2,4,6,6,17,21,21-D_8, 96%) CP 95%
DLM-6896	Pregnenolone (17,21,21,21-D_4, 98%)
CDLM-9158	Pregnenolone (20,21-$^{13}C_2$, 99%;16,16-D_2, 98%)
ULM-9159	Pregnenolone (unlabeled)
CDLM-9160	Pregnenolone sulfate•sodium salt (20,21-$^{13}C_2$, 99%;16,16-D_2, 98%)
ULM-9161	Pregnenolone sulfate•sodium salt (unlabeled)
CLM-457	Progesterone (3,4-$^{13}C_2$, 90%)
CLM-9162	Progesterone (2,3,4-$^{13}C_3$, 99%)
CLM-9162-B	Progesterone (2,3,4-$^{13}C_3$, 99%) 50 µg/mL in acetonitrile
CLM-9162-C	Progesterone (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in acetonitrile
DLM-6909	Progesterone (2,2,6,6,17,21,21,21-D_8, 96%)
DLM-7953	Progesterone (2,2,4,6,6,17α,21,21,21-D_9, 98%)
ULM-8219	Progesterone (unlabeled)
DLM-3312	Prostaglandin A2 (3,3,4,4-D_4, 98%)
DLM-3627	Prostaglandin A2 (3,3,4,4-D_4, 98%) (in solution)
DLM-3728	Prostaglandin E1 (3,3,4,4-D_4, 98%) (in solution)
DLM-3592	Prostaglandin E2 (3,3,4,4-D_4, 98%) 500 µg/mL in methyl acetate
DLM-3628	Prostaglandin E2 (3,3,4,4-D_4, 98%) (in solution)
DLM-3558	Prostaglandin F2α (3,3,4,4-D_4, 98%) (in solution)
DLM-4200	9-α,11-α-Prostaglandin F2 (3,3',4,4'-D_4, 98%) (in solution)
DLM-7457	Sodium 17β-estradiol 3-sulfate (2,4,16,16-D_4, 98%) (stabilized with 50% w/w tris)
DLM-7456	Sodium estrone 3-sulfate (2,4,16,16-D_4, 98%) (stabilized with 50% w/w tris)
CLM-159	Testosterone (3,4-$^{13}C_2$, 99%)
CLM-9164	Testosterone (2,3,4-$^{13}C_3$, 99%)
CLM-9164-C	Testosterone (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in 1,2-dimethoxyethane
CLM-9164-D	Testosterone (2,3,4-$^{13}C_3$, 99%) 1000 µg/mL in 1,2-dimethoxyethane
DLM-683	Testosterone (1,2-D_2, 98%)
DLM-6224	Testosterone (16,16,17-D_3, 98%)
DLM-8085	Testosterone (2,2,4,6,6-D_5, 98%)
ULM-8081	Testosterone (unlabeled)
ULM-8933	Testosterone benzoate (unlabeled)
DLM-8265	Testosterone diacetate (testosterone-D_4, acetate methyl-D_6, 98%)
ULM-9163	3α,5β-Tetrahydroaldosterone (unlabeled)
DLM-7477	3-α,5-β-Tetrahydrodeoxycorticosterone (17,21,21-D_3, 97%) CP 96%
CLM-7185	3,3',5-Triiodo-L-thyronine (ring-$^{13}C_6$, 99%) CP >90%
CLM-6725	L-Thyroxine (tyrosine-ring-$^{13}C_6$, 99%) CP 90%
CLM-8931	L-Thyroxine (ring-$^{13}C_{12}$, 99%) CP 97%
ULM-8184	L-Thyroxine (unlabeled)

Please visit
isotope.com
for new products
and updates

Amino Acids

99% Enriched Amino Acids

Higher enrichment provides improved accuracy in quantitative MS-based proteomic applications. These materials represent the highest isotopically enriched amino acids that are commercially available. These products are shaded in grey throughout the catalog.

Catalog No.	Description
DLM-9219	L-Abrine (methyl-D_3, 98%)
CLM-1655	D-Alanine (1-^{13}C, 99%)
CLM-2495	D-Alanine (3-^{13}C, 99%)
CLM-3229	D-Alanine (2,3-$^{13}C_2$, 99%)
DLM-3545	D-Alanine (2,3,3,3-D_4, 98%)
DLM-7326	D-Alanine (D_7, 98%) (<5% L)
NLM-6762	D-Alanine (^{15}N, 98%)
CLM-705	DL-Alanine (1-^{13}C, 99%)
CLM-115	DL-Alanine (2-^{13}C, 99%)
CLM-707	DL-Alanine (3-^{13}C, 99%)
CLM-6056	DL-Alanine (2,3-$^{13}C_2$, 99%)
CLM-4514	DL-Alanine ($^{13}C_3$, 98%)
DLM-2760	DL-Alanine (2-D, 98%)
DLM-176	DL-Alanine (3,3,3-D_3, 98%)
DLM-1276	DL-Alanine (2,3,3,3-D_4, 97-98%)
NLM-706	DL-Alanine (^{15}N, 98%)
CDLM-8650	DL-Alanine (3-^{13}C, 99%; 2-D, 96%)
CNLM-7839	DL-Alanine (1-^{13}C, 99%; ^{15}N, 98%)
CNLM-3552	DL-Alanine (1,3-$^{13}C_2$, 99%; ^{15}N, 99%)
CLM-116	L-Alanine (1-^{13}C, 99%)
CLM-2016	L-Alanine (2-^{13}C, 99%)
CLM-117	L-Alanine (3-^{13}C, 99%)
CLM-2734	L-Alanine (2,3-$^{13}C_2$, 99%)
CLM-2184-H	L-Alanine ($^{13}C_3$, 99%)
DLM-3101	L-Alanine (2-D, 98%)
DLM-248	L-Alanine (3,3,3-D_3, 99%)
DLM-250	L-Alanine (2,3,3,3-D_4, 98%)
DLM-251	L-Alanine (D_7, 98%)
NLM-454	L-Alanine (^{15}N, 98%)
OLM-7460	L-Alanine ($^{18}O_2$, 90%+)
CDLM-8649	L-Alanine (3-^{13}C, 99%; 2-D, 96%)
CDLM-3439	L-Alanine (3-^{13}C, 99%; 3,3,3-D_3, 98%)
CNLM-6993	L-Alanine (1-^{13}C, 99%; ^{15}N, 98%)
CNLM-3594	L-Alanine (2-^{13}C, 99%; ^{15}N, 98%)
CNLM-534-H	L-Alanine ($^{13}C_3$, 99%; ^{15}N, 99%)
DNLM-7178	L-Alanine (2,3,3,3-D_4, 98%; ^{15}N, 98%)
CDNLM-6800	L-Alanine ($^{13}C_3$, 97-99%; D_4, 97-99%; ^{15}N, 97-99%)
NLM-1656	β-Alanine (^{15}N, 98%+)
CNLM-8457	β-Alanine (1,2-$^{13}C_2$, 99%; ^{15}N, 98%)
CLM-8742	L-*allo*-Isoleucine ($^{13}C_6$, 97-99%)
DLM-857	L-*allo*-Isoleucine (3-methyl-D_3, 98%)
DLM-1505	L-*allo*-Isoleucine (D_{10}, 98%)
CNLM-8670	L-*allo*-Isoleucine ($^{13}C_6$, 97-99%; ^{15}N, 97-99%)
CDNLM-8911	L-*allo*-Isoleucine ($^{13}C_6$, 97-99%; D_{10}, 97-99%; ^{15}N, 97-99%)
CLM-1268	L-Arginine•HCl (1-^{13}C, 99%)
CLM-2070	L-Arginine•HCl (guanido-^{13}C, 99%)
CLM-2051	L-Arginine•HCl (1,2-$^{13}C_2$, 99%)

Catalog No.	Description
CLM-2265-H	L-Arginine•HCl ($^{13}C_6$, 99%)
DLM-6038	L-Arginine•HCl (4,4,5,5-D_4, 94%) (<5% D)
DLM-541	L-Arginine•HCl (D_7, 98%)
NLM-1267	L-Arginine•HCl (α-^{15}N, 98%+)
NLM-395	L-Arginine•HCl (guanido-$^{15}N_2$, 98%+)
NLM-396	L-Arginine•HCl ($^{15}N_4$, 98%)
CDLM-3789	L-Arginine•HCl (5-^{13}C, 99%; 4,4,5,5-D_4, 95%)
CNLM-539-H	L-Arginine•HCl ($^{13}C_6$, 99%; $^{15}N_4$, 99%)
DNLM-7543	L-Arginine•HCl (D_7, 98%; $^{15}N_4$, 98%)
CDNLM-6801	L-Arginine•HCl ($^{13}C_6$, 97-99%; D_7, 97-99%; $^{15}N_4$, 97-99%)
ULM-8347	L-Arginine•HCl (unlabeled)
CLM-8699-H	L-Asparagine•H_2O ($^{13}C_4$, 99%)
DLM-6844	L-Asparagine•H_2O (2,3,3-D_3, 94%)
NLM-2293	L-Asparagine (α-^{15}N, 99%)
NLM-120	L-Asparagine•H_2O (amide-^{15}N, 98%+)
NLM-3286	L-Asparagine•H_2O ($^{15}N_2$, 98%)
CNLM-3819-H	L-Asparagine•H_2O ($^{13}C_4$, 99%; $^{15}N_2$, 99%)
DNLM-6932	L-Asparagine•H_2O (2,3,3-D_3, 98%; $^{15}N_2$, 98%)
CDNLM-6802	L-Asparagine•H_2O ($^{13}C_4$, 97-99%; D_3, 97-99%; $^{15}N_2$, 97-99%)
CLM-864	DL-Aspartic acid (2-^{13}C, 99%)
CLM-518	DL-Aspartic acid (4-^{13}C, 99%)
DLM-832	DL-Aspartic acid (2,3,3-D_3, 98%)
CLM-3616	L-Aspartic acid (1-^{13}C, 99%)
CLM-3617	L-Aspartic acid (2-^{13}C, 99%)
CLM-627	L-Aspartic acid (3-^{13}C, 99%)
CLM-519	L-Aspartic acid (4-^{13}C, 99%) CP 96%
CLM-4455	L-Aspartic acid (1,4-$^{13}C_2$, 99%)
CLM-1801-H	L-Aspartic acid ($^{13}C_4$, 99%)
DLM-546	L-Aspartic acid (2,3,3-D_3, 98%)
NLM-718	L-Aspartic acid (^{15}N, 98%)
CNLM-544-H	L-Aspartic acid ($^{13}C_4$, 99%; ^{15}N, 99%)
DNLM-6931	L-Aspartic acid (2,3,3-D_3, 98%; ^{15}N, 98%)
CDNLM-6803	L-Aspartic acid ($^{13}C_4$, 97-99%; D_3, 97-99%; ^{15}N, 97-99%)
ULM-8676	L-Aspartic acid (unlabeled)
CLM-4899	L-Citrulline (ureido-^{13}C, 99%)
DLM-3860	L-Citrulline (5,5-D_2, 98%)
DLM-6039	L-Citrulline (4,4,5,5-D_4, 95%)
NLM-6850	L-Citrulline (ureido-$^{15}N_1$, 98%)
CDLM-7879	L-Citrulline (ureido-^{13}C, 99%; 5,5-D_2, 98%)
CDLM-8808	L-Citrulline (ureido-^{13}C, 99%; 3,3,4-D_3, 98%)
CDLM-7139	L-Citrulline (5-^{13}C, 99%; 4,4,5,5-D_4, 95%)
ULM-8642	L-Citrulline (unlabeled)
CLM-3790	DL-Cysteine (1-^{13}C, 99%)
CLM-898	DL-Cysteine (3-^{13}C, 99%)
DLM-1180	DL-Cysteine (2-D, 98%)
DLM-899	DL-Cysteine (3,3-D_2, 98%)
CLM-3852	L-Cysteine (1-^{13}C, 99%)

CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

CP = chemical purity

Amino Acids

Catalog No.	Description
CLM-1868	L-Cysteine (3-^{13}C, 99%)
CLM-4320-H	L-Cysteine ($^{13}C_3$, 99%)
DLM-769	L-Cysteine (3,3-D_2, 98%)
DLM-6901	L-Cysteine (2,3,3-D_3, 98%)
NLM-2295	L-Cysteine (^{15}N, 98%)
CNLM-3871-H	L-Cysteine ($^{13}C_3$, 99%; ^{15}N, 99%)
DNLM-6902	L-Cysteine (2,3,3-D_3, 98%; ^{15}N, 98%)
CDNLM-6809	L-Cysteine ($^{13}C_3$, 97-99%; D_3, 97-99%; ^{15}N, 97-99%)
CLM-815	DL-Cystine (3,3'-$^{13}C_2$, 98%)
DLM-1000	DL-Cystine (3,3,3',3'-D_4, 98%)
NLM-1668	DL-Cystine ($^{15}N_2$, 95%)
CLM-520	L-Cystine (3,3'-$^{13}C_2$, 99%)
NLM-3818	L-Cystine ($^{15}N_2$, 98%)
CNLM-4244-H	L-Cystine ($^{13}C_6$, 99%; $^{15}N_2$, 99%)
CDNLM-8659	L-Cystine ($^{13}C_6$, 98%; D_6, 98%; $^{15}N_2$, 98%) CP 95%
CLM-3721	DL-Glutamic acid•H_2O (1-^{13}C, 99%)
CLM-3632	DL-Glutamic acid (3-^{13}C, 99%)
CLM-3028	DL-Glutamic acid (5-^{13}C, 99%)
DLM-335	DL-Glutamic acid (2,4,4-D_3, 98%)
DLM-357	DL-Glutamic acid (2,3,3,4,4-D_5, 97%)
NLM-3279	DL-Glutamic acid (^{15}N, 98%)
CNLM-4292	DL-Glutamic acid•H_2O (1-^{13}C, 99%; ^{15}N, 98%)
ULM-8940	DL-Glutamic acid•H_2O (unlabeled)
CLM-674	L-Glutamic acid (1-^{13}C, 99%)
CLM-2474	L-Glutamic acid (2-^{13}C, 99%)
CLM-4742	L-Glutamic acid (3-^{13}C, 99%)
CLM-2431	L-Glutamic acid (4-^{13}C, 98-99%)
CLM-613	L-Glutamic acid (5-^{13}C, 99%)
CLM-2024	L-Glutamic acid (1,2-$^{13}C_2$, 99%)
CLM-3646	L-Glutamic acid (3,4-$^{13}C_2$, 99%)
CLM-1800-H	L-Glutamic acid ($^{13}C_5$, 99%)
DLM-3725	L-Glutamic acid (2,4,4-D_3, 97-98%)
DLM-556	L-Glutamic acid (2,3,3,4,4-D_5, 97-98%)
NLM-135	L-Glutamic acid (^{15}N, 98%)
OLM-8028	L-Glutamic acid•HCl ($^{17}O_4$, ~30%)
CNLM-554-H	L-Glutamic acid ($^{13}C_5$, 99%; ^{15}N, 99%)
DNLM-6996	L-Glutamic acid (2,3,3,4,4-D_5, 98%; ^{15}N, 98%)
CDNLM-6804	L-Glutamic acid ($^{13}C_5$, 97-99%; D_5, 97-99%; ^{15}N, 97-99%)
ULM-8675	L-Glutamic acid (unlabeled)
CLM-3612	L-Glutamine (1-^{13}C, 99%)
CLM-3613	L-Glutamine (2-^{13}C, 99%)
CLM-770	L-Glutamine (4-^{13}C, 99%)
CLM-1166	L-Glutamine (5-^{13}C, 99%)
CLM-2001	L-Glutamine (1,2-$^{13}C_2$, 99%)
CLM-3641	L-Glutamine (3,4-$^{13}C_2$, 99%)
CLM-1822-H	L-Glutamine ($^{13}C_5$, 99%)
DLM-1826	L-Glutamine (2,3,3,4,4-D_5, 97%)
NLM-1016	L-Glutamine (α-^{15}N, 98%)
NLM-557	L-Glutamine (amide-^{15}N, 98%+)
NLM-1328	L-Glutamine ($^{15}N_2$, 98%)
CNLM-1275-H	L-Glutamine ($^{13}C_5$, 99%; $^{15}N_2$, 99%)
DNLM-6997	L-Glutamine (2,3,3,4,4-D_5, 98%; $^{15}N_2$, 98%)
CDNLM-6805	L-Glutamine ($^{13}C_5$, 97-99%; D_5, 97-99%; $^{15}N_2$, 97-99%)

Catalog No.	Description
CLM-422	Glycine (1-^{13}C, 99%)
CLM-136	Glycine (2-^{13}C, 99%)
CLM-1017	Glycine (1,2-$^{13}C_2$, 97-99%)
DLM-280	Glycine (D_5, 98%)
DLM-1674	Glycine (2,2-D_2, 98%)
NLM-202	Glycine (^{15}N, 98%)
CDLM-6072	Glycine (1-^{13}C, 99%; D_5, 98%)
CNLM-507	Glycine (1-^{13}C, 99%; ^{15}N, 98%+)
CNLM-508	Glycine (2-^{13}C, 99%; ^{15}N, 98%+)
CNLM-1673-H	Glycine ($^{13}C_2$, 99%; ^{15}N, 99%)
DNLM-6862	Glycine (2,2-D_2, 98%; ^{15}N, 98%)
CDNLM-6799	Glycine ($^{13}C_2$, 97-99%; 2,2-D_2, 97-99%; ^{15}N, 97-99%)
CNLM-7175	Glycine•HCl, ethyl ester ($^{13}C_2$, 98%; ^{15}N, 98%)
CLM-2636	DL-Histidine (ring-2-^{13}C, 99%)
CLM-739	DL-Histidine•HCl (ring-2-^{13}C, 99%)
NLM-138	DL-Histidine•2HCl (α-^{15}N, 98%+)
NLM-6703	DL-Histidine•HCl:H_2O (ring-$^{15}N_2$, 98%)
NLM-6702	DL-Histidine•HCl:H_2O ($^{15}N_3$, 98%)
CLM-4793	L-Histidine (carbonyl-^{13}C, 99%)
CLM-1512	L-Histidine•HCl•H_2O (ring-2-^{13}C, 99%) (<5% D)
CLM-2264	L-Histidine•HCl•H_2O ($^{13}C_6$, 97-99%) (<5% D)
NLM-2245	L-Histidine•HCl•H_2O (α-^{15}N, 98%+)
NLM-4457	L-Histidine (ring-π-^{15}N, 98+%) (<5% D)
NLM-846	L-Histidine•HCl•H_2O (ring-π-^{15}N, 98%+) (<5% D)
NLM-4649	L-Histidine (ring-ε-^{15}N, 98%+) (<5% D)
NLM-4765	L-Histidine•HCl•H_2O (ring-$^{15}N_2$, 98%)
NLM-1513	L-Histidine•HCl•H_2O ($^{15}N_3$, 98%) (<5% D)
CNLM-758	L-Histidine•HCl•H_2O ($^{13}C_6$, 97-99%; $^{15}N_3$, 97-99%) (<5% D)
DNLM-7366	L-Histidine•HCl•H_2O (D_5, 98%; $^{15}N_3$, 98%)
CDNLM-6806	L-Histidine•HCl•H_2O ($^{13}C_6$, 97-99%; D_5, 97-99%; $^{15}N_3$, 97-99%)
ULM-8798	L-Histidine (unlabeled)
DLM-2949	τ-methyl-L-Histidine (methyl-D_3, 98%)
CNLM-4645	L-Homoarginine•HCl ($^{13}C_7$, 98%+; $^{15}N_4$, 98%+)
DLM-8259	DL-Homocysteine (3,3,4,4-D_4, 98%) CP 95%+
DLM-3619	DL-Homocystine (3,3,3',3',4,4,4',4'-D_8, 98%)
NLM-2466	L-Homoserine (^{15}N, 95-99%)
CLM-1026	L-Isoleucine (1-^{13}C, 99%)
CLM-2248-H	L-Isoleucine ($^{13}C_6$, 99%)
DLM-1205	L-Isoleucine (3-methyl-D_3, 98%)
DLM-141	L-Isoleucine (D_{10}, 98%)
NLM-292	L-Isoleucine (^{15}N, 98%)
CNLM-561-H	L-Isoleucine ($^{13}C_6$, 99%; ^{15}N, 99%)
DNLM-7325	L-Isoleucine (D_{10}, 98%; ^{15}N, 98%)
CDNLM-4282	L-Isoleucine ($^{13}C_6$, 95-97%; ^{15}N, 96-99%; 2,3-D_2, 97%+)
CDNLM-6807	L-Isoleucine ($^{13}C_6$, 97-99%; D_{10}, 97-99%; ^{15}N, 97-99%)
ULM-8797	L-Isoleucine (unlabeled)
NLM-3585	D-Leucine (^{15}N, 98%)
CLM-204	DL-Leucine (1-^{13}C, 99%)
CLM-207	DL-Leucine (2-^{13}C, 99%)
NLM-355	DL-Leucine (^{15}N, 98%)
CNLM-807	DL-Leucine (1-^{13}C, 99%; ^{15}N, 98%)
CNLM-8679	DL-Leucine (2-^{13}C, 99%; ^{15}N, 98%)

CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

CP = chemical purity

(continued)

Amino Acids

Catalog No.	Description
CLM-468	L-Leucine (1-^{13}C, 99%)
CLM-2014	L-Leucine (2-^{13}C, 99%)
CLM-3524	L-Leucine (1,2-$^{13}C_2$, 99%)
CLM-3383	L-Leucine (3,4-$^{13}C_2$, 99%)
CLM-2262-H	L-Leucine ($^{13}C_6$, 99%)
DLM-1259	L-Leucine (5,5,5-D_3, 99%)
DLM-4212	L-Leucine (isopropyl-D_7, 98%)
DLM-567	L-Leucine (D_{10}, 98%)
NLM-142	L-Leucine (^{15}N, 98%)
OLM-2041	L-Leucine ($^{18}O_2$, 94%)
CNLM-615	L-Leucine (1-^{13}C, 99%; ^{15}N, 98%+)
CNLM-3450	L-Leucine (2-^{13}C, 99%; ^{15}N, 95-99%)
CNLM-3451	L-Leucine (4-^{13}C, 99%; ^{15}N, 99%)
CNLM-281-H	L-Leucine ($^{13}C_6$, 99%; ^{15}N, 99%)
COLM-7399	L-Leucine (1-^{13}C, 99%; $^{17}O_2$, 60%+)
DNLM-1895	L-Leucine (5,5,5-D_3, 98%; ^{15}N, 99%)
DNLM-4642	L-Leucine (D_{10}, 98%; ^{15}N, 97%)
CDNLM-6808	L-Leucine ($^{13}C_6$, 97-99%; D_{10}, 97-99%; ^{15}N, 97-99%)
ULM-8203	L-Leucine (unlabeled)
ULM-4889	D-Lysine•HCl (unlabeled)
CLM-749	DL-Lysine•2HCl (1-^{13}C, 99%)
CLM-7639-H	DL-Lysine•HCl ($^{13}C_6$, 99%)
DLM-8941	DL-Lysine•2HCl (4,4,5,5-D_4, 96-98%)
DLM-3542	DL-Lysine•HCl (3,3,4,4,5,5,6,6-D_8, 98%)
DLM-8089	DL-Lysine•2HCl (D_9, 98%)
NLM-1683	DL-Lysine•HCl•H_2O (α-^{15}N, 99%)
NLM-1031	DL-Lysine•2HCl (ε-^{15}N, 98%)
NLM-8762	DL-Lysine•2HCl ($^{15}N_2$, 98%)
CNLM-3452	DL-Lysine•2HCl (1-^{13}C, 99%; ε-^{15}N, 99%)
CNLM-3453	DL-Lysine•2HCl (2-^{13}C, 99%; ε-^{15}N, 99%)
CNLM-7771-H	DL-Lysine•HCl ($^{13}C_6$, 99%; $^{15}N_2$, 99%)
DNLM-7400	DL-Lysine•2HCl (D_9, 98%; $^{15}N_2$, 98%)
CLM-653	L-Lysine•2HCl (1-^{13}C, 99%)
CLM-633	L-Lysine•HCl (5-^{13}C, 99%)
CLM-632	L-Lysine•2HCl (6-^{13}C, 99%)
CLM-2247-H	L-Lysine•2HCl ($^{13}C_6$, 99%)
DLM-2640	L-Lysine•2HCl (4,4,5,5-D_4, 96-98%)
DLM-2641	L-Lysine•2HCl (3,3,4,4,5,5,6,6-D_8, 98%)
DLM-570	L-Lysine•2HCl (D_9, 98%)
NLM-3529	L-Lysine•HCl•H_2O ($^{14}N_2$, 99.99%)
NLM-143	L-Lysine•2HCl (α-^{15}N, 95-99%)
NLM-631	L-Lysine•2HCl (ε-^{15}N, 98%+)
NLM-1554	L-Lysine•2HCl ($^{15}N_2$, 98%+)
CNLM-291-H	L-Lysine•2HCl ($^{13}C_6$, 99%; $^{15}N_2$, 99%)
DNLM-7545	L-Lysine•2HCl (D_9, 98%; $^{15}N_2$, 98%)
CDNLM-6810	L-Lysine•2HCl ($^{13}C_6$, 97-99%; D_9, 97-99%; $^{15}N_2$, 97-99%)
ULM-8766	L-Lysine•2HCl (unlabeled)
CLM-7356	D-Methionine (1-^{13}C, 99%)
CLM-6191	DL-Methionine (1-^{13}C, 99%)
CLM-3387	DL-Methionine (methyl-^{13}C, 99%)
CLM-6570	DL-Methionine (1,2,3,4-$^{13}C_4$, 99%)
CLM-6571	DL-Methionine ($^{13}C_5$, 99%)

Catalog No.	Description
DLM-2933	DL-Methionine (3,3,4,4-D_4, 98%)
DLM-6795	DL-Methionine (2,3,3,4,4-D_5, 98%)
DLM-9019	DL-Methionine (3,3,4,4-D_4; methyl-D_3, 98%)
DLM-6796	DL-Methionine (2,3,3,4,4-D_5; methyl-D_3, 98%)
NLM-1684	DL-Methionine (^{15}N, 98%)
CNLM-6752	DL-Methionine (1,2,3,4-$^{13}C_4$, 99%; ^{15}N, 98%)
CNLM-6753	DL-Methionine ($^{13}C_5$, 98%; ^{15}N, 98%)
CDNLM-8026	DL-Methionine ($^{13}C_5$, 97-99%; D_8, 97-99%; ^{15}N, 97-99%)
CLM-3267	L-Methionine (1-^{13}C, 99%)
CLM-206	L-Methionine (methyl-^{13}C, 99%)
CLM-893-H	L-Methionine ($^{13}C_5$, 99%)
DLM-431	L-Methionine (methyl-D_3, 98%)
DLM-6797	L-Methionine (2,3,3,4,4-D_5; methyl-D_3, 98%)
NLM-752	L-Methionine (^{15}N, 96-98%)
CDLM-760	L-Methionine (1-^{13}C, 99%; methyl-D_3, 98%)
CDLM-8885	L-Methionine (2,3,3,4,4-D_5, 98%; methyl-$^{13}CH_3$, 99%)
CNLM-759-H	L-Methionine ($^{13}C_5$, 99%; ^{15}N, 99%)
DNLM-7179	L-Methionine (D_8, 98%; ^{15}N, 98%)
CDNLM-6798	L-Methionine ($^{13}C_5$, 97-99%; D_8, 97-99%; ^{15}N, 97-99%)
ULM-8204	L-Methionine (unlabeled)
CLM-8002	L-Methionine sulfone (1-^{13}C, 99%)
CLM-1289	DL-Ornithine•2HCl (1-^{13}C, 99%)
CLM-2176	DL-Ornithine•HCl (1,2-$^{13}C_2$, 99%)
CLM-4219	DL-Ornithine•HCl ($^{13}C_5$, 97-98%)
DLM-200	DL-Ornithine•HCl (3,3,4,4,5,5-D_6, 97-98%)
DLM-6668	DL-Ornithine•HCl (D_7, 98%)
NLM-1047	DL-Ornithine•HCl (α-^{15}N, 98%)
NLM-8064	DL-Ornithine•HCl ($^{15}N_2$, 98%)
CNLM-7577-H	DL-Ornithine•HCl ($^{13}C_5$, 99%; $^{15}N_2$, 99%)
DNLM-8618	DL-Ornithine•HCl (D_7, 98%; 5-^{15}N, 98%)
CLM-3588	L-Ornithine•HCl (1-^{13}C, 99%)
CLM-1036	L-Ornithine•HCl (1,2-$^{13}C_2$, 99%)
CLM-4724-H	L-Ornithine•HCl ($^{13}C_5$, 99%)
DLM-4261	L-Ornithine•HCl (5,5-D_2, 98%)
DLM-6046	L-Ornithine•HCl (4,4,5,5-D_4, 95%)
DLM-2969	L-Ornithine•HCl (3,3,4,4,5,5-D_6, 98%)
DLM-6669	L-Ornithine•HCl (D_7, 98%)
NLM-2212	L-Ornithine•HCl (α-^{15}N, 98%)
NLM-2174	L-Ornithine•HCl (5-^{15}N, 98%)
NLM-3610	L-Ornithine•HCl ($^{15}N_2$, 98%)
CDLM-3873	L-Ornithine•HCl (5-^{13}C, 99%; 4,4,5,5-D_4, 95%)
CNLM-7578-H	L-Ornithine•HCl ($^{13}C_5$, 99%; $^{15}N_2$, 99%)
DNLM-7369	L-Ornithine•HCl (D_7, 98%; $^{15}N_2$, 98%)
ULM-8641	L-Ornithine•HCl (unlabeled)
CLM-3208	D-Phenylalanine (1-^{13}C, 99%)
DLM-4526	D-Phenylalanine (ring-D_5, 97%)
CLM-761	DL-Phenylalanine (1-^{13}C, 99%)
CLM-3268	DL-Phenylalanine (3-^{13}C, 99%)
CLM-7486	DL-Phenylalanine (ring-$^{13}C_6$, 99%)
DLM-2983	DL-Phenylalanine (2-D, 98%)
DLM-2986	DL-Phenylalanine (ring-D_5, 98%)
DLM-2987	DL-Phenylalanine (D_8, 98%)

CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

Amino Acids

Catalog No.	Description
NLM-3434	DL-Phenylalanine (^{15}N, 98%)
CLM-762	L-Phenylalanine (1-^{13}C, 99%)
CLM-1631	L-Phenylalanine (2-^{13}C, 99%) CP 97%
CLM-1053	L-Phenylalanine (3-^{13}C, 99%)
CLM-1055	L-Phenylalanine (ring-$^{13}C_6$, 99%)
CLM-2250-H	L-Phenylalanine ($^{13}C_9$, 99%)
DLM-2984	L-Phenylalanine (2-D, 95%)
DLM-2985	L-Phenylalanine (3,3-D_2, 98%)
DLM-1258	L-Phenylalanine (ring-D_5, 98%)
DLM-2652	L-Phenylalanine (ring-D_5, 3,3-D_2, 98%)
DLM-372	L-Phenylalanine (D_8, 98%)
NLM-108	L-Phenylalanine (^{15}N, 98%)
CNLM-7611	L-Phenylalanine (2,3-$^{13}C_2$, 99%; ^{15}N, 98%)
CNLM-575-H	L-Phenylalanine ($^{13}C_9$, 99%; ^{15}N, 99%)
DNLM-7180	L-Phenylalanine (D_8, 98%; ^{15}N, 98%)
CDNLM-6811	L-Phenylalanine ($^{13}C_9$, 97-99%; D_8, 97-99%; ^{15}N, 97-99%)
CLM-2479	DL-Proline (1-^{13}C, 99%)
DLM-2657	DL-Proline (2,3,3,4,4,5,5-D_7, 97-98%)
NLM-1689	DL-Proline (^{15}N, 98%)
CLM-510	L-Proline (1-^{13}C, 99%)
CLM-2260-H	L-Proline ($^{13}C_5$, 99%)
DLM-487	L-Proline (D_7, 97-98%)
NLM-835	L-Proline (^{15}N, 98%)
CNLM-436-H	L-Proline ($^{13}C_5$, 99%; ^{15}N, 99%)
DNLM-7562	L-Proline (D_7, 98%; ^{15}N, 98%)
CDNLM-6812	L-Proline ($^{13}C_5$, 97-99%; D_7, 97-99%; ^{15}N, 97-99%)
ULM-8333	L-Proline (unlabeled)
CLM-1075	DL-Serine (1-^{13}C, 99%)
CLM-496	DL-Serine (2-^{13}C, 99%)
CLM-497	DL-Serine (3-^{13}C, 99%)
DLM-1074	DL-Serine (3,3-D_2, 98%)
DLM-1073	DL-Serine (2,3,3-D_3, 98%)
NLM-1531	DL-Serine (^{15}N, 98%)
CNLM-4207	DL-Serine ($^{13}C_3$, 98%; ^{15}N, 98%)
ULM-8939	DL-Serine (unlabeled)
CLM-1573	L-Serine (1-^{13}C, 99%)
CLM-2013	L-Serine (2-^{13}C, 99%)
CLM-1572	L-Serine (3-^{13}C, 99%)
CLM-1574-H	L-Serine ($^{13}C_3$, 99%)
DLM-161	L-Serine (3,3-D_2, 98%)
DLM-582	L-Serine (2,3,3-D_3, 98%)
NLM-2036	L-Serine (^{15}N, 98%)
CNLM-3467	L-Serine (2-^{13}C, 99%; ^{15}N, 99%)
CNLM-474-H	L-Serine ($^{13}C_3$, 99%; ^{15}N, 99%)
DNLM-6863	L-Serine (2,3,3-D_3, 98%; ^{15}N, 98%)
CDNLM-6813	L-Serine ($^{13}C_3$, 97-99%; D_3, 97-99%; ^{15}N, 97-99%)
ULM-8799	L-Serine (unlabeled)
CLM-3949	Sodium glutamate•½H_2O ($^{13}C_5$, 97-98%)
DLM-8738	*S*-Sulfo-DL-cysteine (2,3,3-D_3, 99%)
CLM-447	L-Threonine (1-^{13}C, 99%)
CLM-2261-H	L-Threonine ($^{13}C_4$, 99%)
DLM-1693	L-Threonine (D_5, 98%)
NLM-742	L-Threonine (^{15}N, 98%)
CNLM-587	L-Threonine ($^{13}C_4$, 97-99%; ^{15}N, 97-99%)
DNLM-7367	L-Threonine (D_5, 97%; ^{15}N, 98%)
CDNLM-6814	L-Threonine ($^{13}C_4$, 97-99%; D_5, 97-99%; ^{15}N, 97-99%)
ULM-8800	L-Threonine (unlabeled)
CLM-778	L-Tryptophan (1-^{13}C, 99%)
CLM-1543	L-Tryptophan (indole-2-^{13}C, 98%)
CLM-716	L-Tryptophan (indole-3-^{13}C, 95-99%)
CLM-717	L-Tryptophan (indole-4-^{13}C, 99%) CP 95%+
CLM-1301	L-Tryptophan (ring-$^{13}C_6$, 99%)
CLM-4290-H	L-Tryptophan ($^{13}C_{11}$, 99%)
DLM-1092	L-Tryptophan (indole-D_5, 98%)
DLM-6903	L-Tryptophan (D_8, 98%)
NLM-1695	L-Tryptophan (α-^{15}N, 95-99%)
NLM-1208	L-Tryptophan (indole-^{15}N, 98%+)
NLM-800	L-Tryptophan ($^{15}N_2$, 98%)
CNLM-2475-H	L-Tryptophan ($^{13}C_{11}$, 99%; $^{15}N_2$, 99%)
DNLM-6904	L-Tryptophan (D_8, 98%; $^{15}N_2$, 98%)
CDNLM-6816	L-Tryptophan ($^{13}C_{11}$, 97-99%; D_8, 97-99%; $^{15}N_2$, 97-99%)
CLM-448	DL-Tyrosine (1-^{13}C, 99%)
DLM-137	DL-Tyrosine (3,3-D_2, 98%)
DLM-2914	DL-Tyrosine (ring-3,5-D_2, 98%)
CLM-776	L-Tyrosine (1-^{13}C, 99%)
CLM-437	L-Tyrosine (2-^{13}C, 99%)
CLM-3378	L-Tyrosine (3-^{13}C, 99%)
CLM-622	L-Tyrosine (phenol-4-^{13}C, 95-99%)
CLM-623	L-Tyrosine (phenol-3,5-$^{13}C_2$, 95-99%)
CLM-1542	L-Tyrosine (ring-$^{13}C_6$, 99%)
CLM-7881	L-Tyrosine (1-^{13}C, ring-$^{13}C_6$, 99%)
CLM-2263-H	L-Tyrosine ($^{13}C_9$, 99%)
DLM-2317	L-Tyrosine (3,3-D_2, 98%)
DLM-2917	L-Tyrosine (ring-2,6-D_2, 2-D, 98%)
DLM-449	L-Tyrosine (ring-3,5-D_2, 98%)
DLM-451	L-Tyrosine (ring-D_4, 98%)
DLM-589	L-Tyrosine (D_7, 98%)
NLM-590	L-Tyrosine (^{15}N, 98%)
OLM-621	L-Tyrosine (phenol-^{17}O, 35-40%)
OLM-8696	L-Tyrosine (phenol-^{18}O, 85-90%)
CNLM-7610	L-Tyrosine (2,3-$^{13}C_2$, 99%; ^{15}N, 98%)
CNLM-439-H	L-Tyrosine ($^{13}C_9$, 99%; ^{15}N, 99%)
DNLM-7373	L-Tyrosine (D_7, 98%; ^{15}N, 98%)
CDNLM-6815	L-Tyrosine ($^{13}C_9$, 97-99%; D_7, 97-99%; ^{15}N, 97-99%)
ULM-8201	L-Tyrosine (unlabeled)
CLM-7103	3-Chloro-L-tyrosine (ring-$^{13}C_6$, 99%) CP 95%+
CLM-7104	3-Nitro-L-tyrosine (ring-$^{13}C_6$, 99%) CP 94%+
CLM-166	DL-Valine (1-^{13}C, 99%)
CLM-3277	DL-Valine (2-^{13}C, 99%)
DLM-3340	DL-Valine (2-D, 98%)
DLM-3341	DL-Valine (3-D, 98%)
DLM-3342	DL-Valine (2,3-D_2, 98%)
DLM-3675	DL-Valine (dimethyl-D_6, 98%)
DLM-311	DL-Valine (D_8, 98%)
NLM-236	DL-Valine (^{15}N, 98%)

(continued)

Amino Acids

Catalog No.	Description
CNLM-8677	DL-Valine (2-^{13}C, 99%; ^{15}N, 98%)
CLM-470	L-Valine (1-^{13}C, 99%)
CLM-3050	L-Valine (2-^{13}C, 99%)
CLM-2249-H	L-Valine ($^{13}C_5$, 99%)
DLM-7732	L-Valine (3-D, 98%)
DLM-4364	L-Valine (2,3-D_2, 98%)
DLM-488	L-Valine (D_8, 98%)
NLM-316	L-Valine (^{15}N, 98%)
CNLM-3466	L-Valine (1-^{13}C, 99%; ^{15}N, 98%)
CNLM-8678	L-Valine (2-^{13}C, 99%; ^{15}N, 98%)
CNLM-442-H	L-Valine ($^{13}C_5$, 99%; ^{15}N, 99%)
DNLM-4643	L-Valine (D_8, 97%; ^{15}N, 97%)
CDNLM-6817	L-Valine ($^{13}C_5$, 97-99%; D_8, 97-99%; ^{15}N, 97-99%)
ULM-8202	L-Valine (unlabeled)

Large-scale bioreactor commonly used for synthesis of amino acids.

Amino Acid Mixes for Cell-Free Protein Expression

Catalog No.	Description
CLM-1548	Algal amino acid mixture (16AA) (U-^{13}C, 97-99%)
DLM-2082	Algal amino acid mixture (16AA) (U-D, 98%)
NLM-2161	Algal amino acid mixture (16AA) (U-^{15}N, 98%)
CNLM-452	Algal amino acid mixture (16AA) (U-^{13}C, 97-99%; U-^{15}N, 97-99%)
DNLM-819	Algal amino acid mixture (16AA) (U-D, 98%; U-^{15}N, 98%)
CDNLM-2496	Algal amino acid mixture (16AA) (U-^{13}C, 97-99%; U-D, 97-99%; U-^{15}N, 97-99%)
ULM-2314	Algal amino acid mixture (16AA) (unlabeled)

Catalog No.	Description
DLM-6819	"Cell Free" amino acid mix (20AA) (U-D, 98%)
NLM-6695	"Cell Free" amino acid mix (20AA) (U-^{15}N, 96-98%)
CNLM-6696	"Cell Free" amino acid mix (20AA) (U-^{13}C, 97-99%+; U-^{15}N, 97-99%)
DNLM-6818	"Cell Free" amino acid mix (20AA) (U-D, 98%; U-^{15}N, 98%)
CDNLM-6784	"Cell Free" amino acid mix (20AA) (U-^{13}C, 97-99%; U-^{15}N, 97-99%; U-D, 97-99%)
ULM-7891	"Cell Free" amino acid mix (20AA) (unlabeled)

CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

Protected Amino Acids

Catalog No.	Description
NLM-3289	D-Alanine-*N*-acetyl (^{15}N, 98%)
NLM-2168	D-Alanine-*N*-*t*-BOC (^{15}N, 98%)
CLM-6599	DL-Alanine-*N*-acetyl (1-^{13}C, 99%)
CLM-7785	L-Alanine-*N*-FMOC ($^{13}C_3$, 97-99%)
CLM-818	L-Alanine-*N*-FMOC (1-^{13}C, 99%)
CLM-3638	L-Alanine-*N*-FMOC (2-^{13}C, 99%)
CLM-1142	L-Alanine-*N*-FMOC (3-^{13}C, 99%)
CLM-2150	L-Alanine-*N*-*t*-BOC (1-^{13}C, 99%)
CLM-2011	L-Alanine-*N*-*t*-BOC (2-^{13}C, 98-99%)
CLM-2151	L-Alanine-*N*-*t*-BOC (3-^{13}C, 99%)
CLM-3589	L-Alanine-*N*-*t*-BOC ($^{13}C_3$, 97-99%)
DLM-7316	L-Alanine-*N*-FMOC (3,3,3-D_3, 98%)
DLM-8168	L-Alanine-*N*-FMOC (2,3,3,3-D_4, 98%)
DLM-649	L-Alanine-*N*-*t*-BOC (2-D_1, 98%)
DLM-2793	L-Alanine-*N*-*t*-BOC (3,3,3-D_3, 99%)
NLM-614	L-Alanine-*N*-FMOC (^{15}N, 98%)
NLM-1903	L-Alanine-*N*-*t*-BOC (^{15}N, 98%)
CNLM-4355	L-Alanine-*N*-FMOC ($^{13}C_3$, 97-99%; ^{15}N, 97-99%)
CNLM-4355-H	L-Alanine-*N*-FMOC ($^{13}C_3$, 99%; ^{15}N, 99%)
CNLM-6014	L-Alanine-*N*-*t*-BOC (2-^{13}C, 99%; ^{15}N, 96-99%)
CNLM-2394	L-Alanine-*N*-*t*-BOC ($^{13}C_3$, 97-99%; ^{15}N, 97-99%)
CDNLM-7852	L-Alanine-*N*-FMOC ($^{13}C_3$, 97-99%; D_4, 97-99%; ^{15}N, 97-99%)
CLM-8475-H	L-Arginine-*N*-FMOC, PBF ($^{13}C_6$, 99%) (contains solvent)
NLM-8841	L-Arginine-*N*-FMOC, PBF ($^{15}N_4$, 98%) (contains solvent)
CNLM-8474-H	L-Arginine-*N*-FMOC, PBF ($^{13}C_6$, 99%; $^{15}N_4$, 99%) (contains solvent)
NLM-2164	L-Asparagine-*N*-*t*-BOC (α-^{15}N, 98%)
NLM-4204	L-Asparagine-*N*-FMOC, β-*N*-trityl ($^{15}N_2$, 98%)
CNLM-6193	L-Asparagine-*N*-FMOC, β-*N*-trityl ($^{13}C_4$, 97-99%; $^{15}N_2$, 97-99%)
CNLM-6193-H	L-Asparagine-*N*-FMOC, β-*N*-trityl ($^{13}C_4$, 99%; $^{15}N_2$, 99%)
DLM-8599	DL-Aspartic acid-*N*-acetyl (aspartate-2,3,3-D_3, 97%)
CLM-4249	L-Aspartic acid-*N*-carboxylbenzyl ($^{13}C_4$, 97-99%)
NLM-647	L-Aspartic acid-*N*-FMOC, β-*O*-*t*-butyl ester (^{15}N, 98%)
NLM-3493	L-Aspartic acid-*N*-*t*-BOC (^{15}N, 98%)
NLM-1908	L-Aspartic acid-*N*-*t*-BOC, β-benzyl ester (^{15}N, 98%)
CNLM-2392	L-Aspartic acid-*N*-*t*-BOC, β-benzyl ester ($^{13}C_4$, 97-99%; ^{15}N, 97-99%)
CNLM-4789	L-Aspartic acid-*N*-FMOC, α-*O*-*t*-butyl ester ($^{13}C_4$, 97-99%;^{15}N, 97-99%)
CNLM-4752	L-Aspartic acid-*N*-FMOC, β-*O*-*t*-butyl ester ($^{13}C_4$, 97-99%; ^{15}N, 97-99%)
CNLM-4752-H	L-Aspartic acid-*N*-FMOC, β-*O*-*t*-butyl ester ($^{13}C_4$, 99%; ^{15}N, 99%)
CNLM-4788	L-Aspartic acid-*N*-FMOC ($^{13}C_4$, 97-99%; ^{15}N, 97-99%)
CLM-2403	L-Cysteine-*N*-FMOC, *S*-benzyl (3-^{13}C, 98%)
CLM-1901	L-Cysteine-*N*-*t*-BOC, *S*-benzyl (3-^{13}C, 99%)
DLM-4721	L-Cysteine-*N*-FMOC, *S*-trityl (3,3-D_2, 98%)
DLM-2700	L-Cysteine-*N*-*t*-BOC, *S*-*p*-methyl benzoate (3,3-D_2, 98%)
DLM-4532	L-Cysteine-*S*-trityl (3,3-D_2, 98%)
DLM-2942	L-Cysteine-*S*-methyl (*S*-methyl-D_3, 98%)
NLM-3874	L-Cysteine-*N*-*t*-BOC, *S*-*p*-methyl benzoate (^{15}N, 98%)
CNLM-7579	L-Cysteine-*N*-acetyl (cysteine-$^{13}C_3$, 97-99%; ^{15}N, 97-99%) CP 95%
CNLM-4722	L-Cysteine-*N*-FMOC, *S*-trityl ($^{13}C_3$, 97-99%; ^{15}N, 97-99%)
CNLM-4722-H	L-Cysteine-*N*-FMOC, *S*-trityl ($^{13}C_3$, 99%; ^{15}N, 99%)

Catalog No.	Description
CLM-6664	L-Glutamic acid-*N*-acetyl (glutamate-$^{13}C_5$, 97-99%)
CLM-2008	L-Glutamic acid-*N*-*t*-BOC, γ-benzyl ester (1,2-$^{13}C_2$, 99%)
NLM-1907	L-Glutamic acid-*N*-*t*-BOC, γ-benzyl ester (^{15}N, 98%)
NLM-8960	L-Glutamic acid-*N*-FMOC, γ-*t*-butyl ester (^{15}N, 98%)
CNLM-4753	L-Glutamic acid-*N*-FMOC, γ-*t*-butyl ester ($^{13}C_5$, 97-99%; ^{15}N, 97-99%) CP 96%
CNLM-4753-H	L-Glutamic acid-*N*-FMOC, γ-*t*-butyl ester ($^{13}C_5$, 99%; ^{15}N, 99%) CP 96%
NLM-3419	L-Glutamine-*N*-*t*-BOC (α-^{15}N, 98%+)
CNLM-4356	L-Glutamine-*N*-FMOC ($^{13}C_5$, 97-99%; $^{15}N_2$, 97-99%)
CNLM-4356-H	L-Glutamine-*N*-FMOC ($^{13}C_5$, 99%; $^{15}N_2$, 99%)
CNLM-7252	L-Glutamine-*N*-FMOC, γ-*N*-trityl ($^{13}C_5$, 97-99%; $^{15}N_2$, 97-99%)
CNLM-7252-H	L-Glutamine-*N*-FMOC, γ-*N*-trityl ($^{13}C_5$, 99%; $^{15}N_2$, 99%)
CLM-2152	Glycine-*N*-*t*-BOC (1-^{13}C, 99%)
CLM-1900	Glycine-*N*-*t*-BOC (2-^{13}C, 99%)
CLM-3639	Glycine-*N*-FMOC (1-^{13}C, 99%)
CLM-2387	Glycine-*N*-FMOC (2-^{13}C, 99%)
CLM-3777	Glycine-*N*-acetyl (2-^{13}C, 99%)
CLM-7547	Glycine-*N*-FMOC ($^{13}C_2$, 97-99%)
DLM-2153	Glycine-*N*-*t*-BOC (2,2-D_2, 98%)
DLM-7339	Glycine-*N*-FMOC (2,2-D_2, 98%)
DLM-2947	Glycine-*N*-methyl (*N*-methyl-D_3, 98%)
NLM-694	Glycine-*N*-FMOC (^{15}N, 98%)
NLM-2109	Glycine-*N*-*t*-BOC (^{15}N, 98%)
NLM-2377	Glycine-*N*-benzoyl (Hippuric acid) (^{15}N, 98%)
NLM-4464	Glycine-*N*-acetyl (^{15}N, 98%)
CNLM-2412	Glycine-*N*-*t*-BOC ($^{13}C_2$, 97-99%; ^{15}N, 97-99%)
CNLM-4357	Glycine-*N*-FMOC ($^{13}C_2$, 97-99%; ^{15}N, 97-99%)
CNLM-4357-H	Glycine-*N*-FMOC ($^{13}C_2$, 99%; ^{15}N, 99%)
CNLM-4524	Glycine-*N*-acetyl ($^{13}C_2$, 97-99%; ^{15}N, 97-99%)
CNLM-7697	Glycine-*N*-FMOC (1-^{13}C, 99%;^{15}N, 98%)
CNLM-7698	Glycine-*N*-FMOC (2-^{13}C, 99%;^{15}N, 98%)
CDNLM-7853	Glycine-*N*-FMOC ($^{13}C_2$, 97-99%; 2,2-D_2, 97-99%; ^{15}N, 97-99%)
CLM-8043	L-Isoleucine-*N*-FMOC (1-^{13}C, 99%)
CLM-7794	L-Isoleucine-*N*-FMOC ($^{13}C_6$, 97-99%)
NLM-391	L-Isoleucine-*N*-FMOC (^{15}N, 98%)
NLM-2167	L-Isoleucine-*N*-*t*-BOC (^{15}N, 98%)
CNLM-4346	L-Isoleucine-*N*-FMOC ($^{13}C_6$, 97-99%; ^{15}N, 97-99%)
CNLM-4346-H	L-Isoleucine-*N*-FMOC ($^{13}C_6$, 99%; ^{15}N, 99%)
CLM-2155	L-Leucine-*N*-*t*-BOC•H_2O (1-^{13}C, 99%)
CLM-2010	L-Leucine-*N*-*t*-BOC•H_2O (2-^{13}C, 99%)
CLM-3691	L-Leucine-*N*-FMOC (1-^{13}C, 99%)
CLM-7546	L-Leucine-*N*-FMOC (1,2-$^{13}C_2$, 99%)
CLM-3683	L-Leucine-*N*-FMOC ($^{13}C_6$, 97-99%)
DLM-2736	L-Leucine-*N*-*t*-BOC•H_2O (5,5,5-D_3, 98%)
DLM-7202	L-Leucine-*N*-FMOC (5,5,5-D_3, 98%)
DLM-476	L-Leucine-*N*-acetyl (D_{10}, 98%)
DLM-7575	L-Leucine-*N*-FMOC (D_{10}, 98%)
DLM-3650	L-Leucine-*N*-*t*-BOC•H_2O (D_{10}, 98%)
NLM-1904	L-Leucine-*N*-*t*-BOC•H_2O (^{15}N, 98%)
NLM-2397	L-Leucine-*N*-FMOC (^{15}N, 98%)
CNLM-2396	L-Leucine-*N*-*t*-BOC•H_2O ($^{13}C_6$, 97-99%; ^{15}N, 97-99%)
CNLM-4345	L-Leucine-*N*-FMOC ($^{13}C_6$, 97-99%; ^{15}N, 97-99%)

(continued)

Protected Amino Acids

Catalog No.	Description
CNLM-4345-H	L-Leucine-*N*-FMOC ($^{13}C_6$, 99%; ^{15}N, 99%)
CDNLM-7854	L-Leucine-*N*-FMOC ($^{13}C_6$, 97-99%; D_{10}, 97-99%; ^{15}N, 97-99%)
CLM-343	L-Lysine-α-*N*-*t*-BOC (6-^{13}C, 99%)
CLM-2302	L-Lysine-α-*N*-*t*-BOC, ε-*N*-2Cl-carboxylbenzyl (6-^{13}C, 99%)
CLM-6194	L-Lysine-α-*N*-FMOC, ε-*N*-*t*-BOC (1-^{13}C, 99%)
CLM-7865-H	L-Lysine-α-*N*-FMOC, ε-*N*-*t*-BOC ($^{13}C_6$, 99%)
DLM-4731	L-Lysine-ε-*N*-carboxylmethyl (4,4,5,5-D_4, 96-98%)
NLM-4631	L-Lysine-α-*N*-FMOC, ε-*N*-*t*-BOC ($^{15}N_2$, 96-98%)
CNLM-4754-H	L-Lysine-α-*N*-FMOC, ε-*N*-*t*-BOC ($^{13}C_6$, 99%; $^{15}N_2$, 99%)
CLM-2156	L-Methionine-*N*-*t*-BOC (methyl-^{13}C, 98%)
CLM-8166	L-Methionine-*N*-FMOC (1-^{13}C, 99%)
NLM-4632	L-Methionine-*N*-FMOC (^{15}N, 98%) CP 95%
CNLM-4358	L-Methionine-*N*-FMOC ($^{13}C_5$, 97-99%; ^{15}N, 97-99%)
CNLM-4358-H	L-Methionine-*N*-FMOC ($^{13}C_5$, 99%; ^{15}N, 99%)
CLM-4824	L-Phenylalanine-*N*-FMOC (1-^{13}C, 99%)
CLM-3684	L-Phenylalanine-*N*-FMOC (ring-$^{13}C_6$, 99%)
CLM-2170	L-Phenylalanine-*N*-*t*-BOC (1-^{13}C, 99%)
CLM-2009	L-Phenylalanine-*N*-*t*-BOC (2-^{13}C, 99%)
CLM-2061	L-Phenylalanine-*N*-*t*-BOC (ring-$^{13}C_6$, 99%)
CLM-7859	L-Phenylalanine-*N*-*t*-BOC ($^{13}C_9$, 97-99%)
DLM-7786	L-Phenylalanine-*N*-FMOC (ring-D_5, 98%)
DLM-8752	L-Phenylalanine-*N*-FMOC (D_8, 98%)
DLM-2157	L-Phenylalanine-*N*-*t*-BOC (ring-D_5, 98%)
NLM-1265	L-Phenylalanine-*N*-FMOC (^{15}N, 98%)
NLM-1905	L-Phenylalanine-*N*-*t*-BOC (^{15}N, 98%)
CNLM-4362	L-Phenylalanine-*N*-FMOC ($^{13}C_9$, 99%; ^{15}N, 99%)
CNLM-4362-H	L-Phenylalanine-*N*-FMOC ($^{13}C_9$, 99%; ^{15}N, 99%)
CNLM-2393	L-Phenylalanine-*N*-*t*-BOC ($^{13}C_9$, 97-99%; ^{15}N, 97-99%)
CLM-8044	L-Proline-*N*-FMOC (1-^{13}C, 99%)
NLM-3906	L-Proline-*N*-FMOC (^{15}N, 98%)
NLM-2329	L-Proline-*N*-*t*-BOC (^{15}N, 96%)
CNLM-4347	L-Proline-*N*-FMOC ($^{13}C_5$, 97-99%; ^{15}N, 99%)
CNLM-4347-H	L-Proline-*N*-FMOC ($^{13}C_5$, 99%; ^{15}N, 97-99%)

Catalog No.	Description
CLM-8167	L-Serine-*N*-FMOC, *O*-*t*-butyl ether (1-^{13}C, 99%)
CLM-2007	L-Serine-*N*-*t*-BOC, *O*-benzyl ether (2-^{13}C, 99%)
CLM-756	L-Serine-*N*-*t*-BOC, *O*-benzyl ether (3-^{13}C, 99%)
NLM-4630	L-Serine-*N*-FMOC, *O*-*t*-butyl ether (^{15}N, 98%)
NLM-2025	L-Serine-*N*-*t*-BOC, *O*-benzyl ether (^{15}N, 98%)
CNLM-4755	L-Serine-*N*-FMOC, *O*-*t*-butyl ether ($^{13}C_3$, 97-99%; ^{15}N, 97-99%)
CNLM-4755-H	L-Serine-*N*-FMOC, *O*-*t*-butyl ether ($^{13}C_3$, 99%; ^{15}N, 99%)
CNLM-8403	L-Serine-*N*-FMOC ($^{13}C_3$, 97-99%; ^{15}N, 97-99%)
CNLM-8403-H	L-Serine-*N*-FMOC ($^{13}C_3$, 99%; ^{15}N, 99%)
NLM-8170	L-Threonine-*N*-FMOC, *O*-*t*-butyl ether (^{15}N, 98%)
NLM-3681	L-Threonine-*N*-*t*-BOC, *O*-benzyl ether (^{15}N, 98%)
CNLM-7615	L-Threonine-*N*-FMOC, *O*-*t*-butyl ether ($^{13}C_4$, 97-99%;^{15}N, 97-99%)
CNLM-7615-H	L-Threonine-*N*-FMOC, *O*-*t*-butyl ether ($^{13}C_4$, 99%;^{15}N, 99%)
CLM-2194	L-Tryptophan-*N*-*t*-BOC (1-^{13}C, 99%)
DLM-6113	L-Tryptophan-*N*-FMOC (indole-D_5, 98%)
NLM-3423	L-Tryptophan-*N*-FMOC (α-^{15}N, 98%+)
CNLM-6077	L-Tryptophan-*N*-FMOC ($^{13}C_{11}$, 97-99%; $^{15}N_2$, 97-99%)
DLM-2303	L-Tyrosine-*N*-*t*-BOC, *O*-benzyl ether (ring-D_4, 98%)
NLM-1906	L-Tyrosine-*N*-*t*-BOC, *O*-benzyl ether (^{15}N, 98%)
NLM-8169	L-Tyrosine-*N*-FMOC, *O*-*t*-butyl ether (^{15}N, 98%)
CNLM-4349	L-Tyrosine-*N*-FMOC, *O*-*t*-butyl ether ($^{13}C_9$, 97-99%; ^{15}N, 97-99%)
CNLM-4349-H	L-Tyrosine-*N*-FMOC, *O*-*t*-butyl ether ($^{13}C_9$, 99%; ^{15}N, 99%)
CLM-2450	D-Valine-*N*-acetyl (2-^{13}C, 99%)
CLM-3640	L-Valine-*N*-FMOC (1-^{13}C, 99%)
CLM-7793	L-Valine-*N*-FMOC ($^{13}C_5$, 97-99%)
CLM-2158	L-Valine-*N*-*t*-BOC (1-^{13}C, 99%)
DLM-7784	L-Valine-*N*-FMOC (D_8, 98%)
DLM-3651	L-Valine-*N*-*t*-BOC (D_8, 98%)
NLM-7888	L-Valine-*N*-acetyl (^{15}N, 98%)
NLM-4243	L-Valine-*N*-FMOC (^{15}N, 98%)
NLM-2060	L-Valine-*N*-*t*-BOC (^{15}N, 98%)
CNLM-4348	L-Valine-*N*-FMOC ($^{13}C_5$, 97-99%; ^{15}N, 97-99%)
CNLM-4348-H	L-Valine-*N*-FMOC ($^{13}C_5$, 99%; ^{15}N, 99%)
CNLM-2395	L-Valine-*N*-*t*-BOC ($^{13}C_5$, 97-99%; ^{15}N, 97-99%)

Preloaded Resins

Catalog No.	Description
SRPR-ARG-CN	L-Arginine-*N*-Pbf ($^{13}C_6$, 99%; $^{15}N_4$, 99%) – 2-ClTrt resin
SRPR-LYS-CN	L-Lysine-*N*-*t*-BOC ($^{13}C_6$, 99%; $^{15}N_2$, 99%) – 2-ClTrt resin

CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

Carbohydrates

Catalog No.	Description
CLM-1220	*N*-Acetylglucosamine (*N*-acetyl-1-^{13}C, 99%)
CLM-1827	*N*-Acetylglucosamine ($^{13}C_6$, 99%)
NLM-8810	*N*-Acetylglucosamine (^{15}N, 98%)
CLM-7642	D-Arabinitol (U-$^{13}C_5$, 98%+)
CLM-715	D-Arabinose (1-^{13}C, 99%)
CLM-1288	D-Arabinose (2-^{13}C, 98%)
CLM-8477	D-Arabinose (U-$^{13}C_5$, 99%)
DLM-1379	D-Arabinose (2-D, 98%)
CLM-1824	2-Deoxy-D-glucose (1-^{13}C, 99%)
CLM-2122	2-Deoxy-D-glucose (6-^{13}C, 99%)
DLM-6732	2-Deoxy-D-glucose (1-D, 98%)
DLM-6940	2-Deoxy-D-glucose (D_8, 98%) CP 88%
CLM-7266	2-Deoxy-D-ribose (1-^{13}C, 99%)
DLM-4750	2-Deoxy-D-ribose (5,5-D_2, 98%)
CLM-3867	Epilactose (1-^{13}C, 98%+)
CLM-1118	D-Erythrose (1-^{13}C, 99%) 1.2% in H_2O
CLM-1387	D-Erythrose (2-^{13}C, 99%) 1.2% in H_2O
CLM-8944	D-Erythrose (4-^{13}C, 99%) 1.2% in H_2O
CLM-7863	D-Erythrose (U-$^{13}C_4$, 98%) 1.2% in H_2O
CLM-1201	D-Fructose (1-^{13}C, 99%)
CLM-1527	D-Fructose (2-^{13}C, 99%)
CLM-7660	D-Fructose (3-^{13}C, 99%)
CLM-7661	D-Fructose (4-^{13}C, 99%)
CLM-7662	D-Fructose (5-^{13}C, 99%)
CLM-1388	D-Fructose (6-^{13}C, 99%)
CLM-528	D-Fructose (1,2-$^{13}C_2$, 99%)
CLM-2462	D-Fructose (1,6-$^{13}C_2$, 99%)
CLM-8415	D-Fructose (1,2,3-$^{13}C_3$, 99%)
CLM-1553	D-Fructose (U-$^{13}C_6$, 99%)
DLM-1389	D-Fructose (6,6-D_2, 98%)
DLM-6050	D-Fructose (1-D, 98%)
CLM-3705	L-Fructose (1'-^{13}C, 99%)
CLM-219	L-Fructose (6-^{13}C, 99%)
CLM-6678	D-Fructose-1,6-bisphosphate, sodium salt (hydrate) (1-^{13}C, 99%)
CLM-8962	D-Fructose-1,6-bisphosphate, disodium salt (hydrate) (U-$^{13}C_6$, 98%)
CLM-8616	D-Fructose-6-phosphate, disodium salt (hydrate) (U-$^{13}C_6$, 99%)
CLM-744	D-Galactose (1-^{13}C, 99%)
CLM-745	D-Galactose (2-^{13}C, 99%)
CLM-4217	D-Galactose (1,2-$^{13}C_2$, 99%)
CLM-1570	D-Galactose (U-$^{13}C_6$, 99%)
DLM-1390	D-Galactose (1-D, 98%)
DLM-1391	D-Galactose (2-D, 98%)
CLM-8998	D-Galactose-1-phosphate, dipotassium salt (1-^{13}C, 99%)
CLM-420	D-Glucose (1-^{13}C, 98-99%)
CLM-746	D-Glucose (2-^{13}C, 99%)
CLM-1393	D-Glucose (3-^{13}C, 99%)
CLM-1394	D-Glucose (4-^{13}C, 99%)
CLM-1395	D-Glucose (5-^{13}C, 98%)
CLM-481	D-Glucose (6-^{13}C, 99%)

Catalog No.	Description
CLM-504	D-Glucose (1,2-$^{13}C_2$, 99%)
CLM-8942	D-Glucose (2,3-$^{13}C_2$, 99%)
CLM-2717	D-Glucose (1-^{13}C, 99%; 6-^{13}C, 97%+)
CLM-6750	D-Glucose (3,4-$^{13}C_2$, 99%)
CLM-8787	D-Glucose (4,5-$^{13}C_2$, 99%)
CLM-4673	D-Glucose (1,2,3-$^{13}C_3$, 99%)
CLM-8770	D-Glucose (4,5,6-$^{13}C_3$, 98%)
CLM-8946	D-Glucose (2,3,4,5,6-$^{13}C_5$, 99%)
CLM-1396	D-Glucose (U-$^{13}C_6$, 99%)
CLM-4819	D-Glucose (U-$^{12}C_6$, 99.9%)
DLM-1150	D-Glucose (1-D, 98%)
DLM-1271	D-Glucose (2-D, 98%)
DLM-3557	D-Glucose (3-D_1, 98%)
DLM-6754	D-Glucose (5-D, 98%)
DLM-349	D-Glucose (6,6-D_2, 99%)
DLM-2062	D-Glucose (1,2,3,4,5,6,6-D_7, 97-98%)
DLM-9047	D-Glucose (U-D_{12}, 98%)
CDLM-6064	D-Glucose (1-^{13}C, 99%; 1-D, 98%)
CDLM-999	D-Glucose (1-^{13}C, 99%; 2-D, 98%)
CDLM-4895	D-Glucose (1-^{13}C, 99%; 6-^{13}C, 97%+; 6,6-D_2, 98%)
CDLM-3813	D-Glucose (U-$^{13}C_6$, 99%; 1,2,3,4,5,6,6-D_7, 97-98%)
CLM-1966	L-Glucose (1-^{13}C, 99%)
CLM-1399	L-Glucose (2-^{13}C, 99%)
CLM-8813	D-Glucose-1-phosphate, dicyclohexylammonium salt monohydrate (U-$^{13}C_6$, 99%+)
CLM-8367	D-Glucose-6-phosphate, disodium salt (hydrate) (U-$^{13}C_6$, 99%+)
DLM-3856	Methyl-D-glucopyranoside (*O*-methyl-D_3, glucose-D_7, 98%)
DLM-7826	*myo*-Inositol (2-D_1, 91%)
CLM-4423	Lactose•H_2O (glucose-$^{13}C_6$, 98%+)
CLM-4518	Lactose ureide•$2H_2O$ (ureide-^{13}C, 99%)
ULM-4519	Lactose ureide•$2H_2O$ (unlabeled)
CLM-1127	D-Lyxose (1-^{13}C, 99%)
CLM-1525	D-Lyxose (2-^{13}C, 99%)
CLM-1128	D-Lyxose (5-^{13}C, 99%)
DLM-1187	D-Lyxose (1-D, 98%)
DLM-1188	D-Lyxose (2-D, 98%)
CLM-2470	L-Lyxose (1,2-$^{13}C_2$, 99%)
CLM-2642	D-Maltose•H_2O (U-$^{13}C_{12}$, 99%)
CLM-1189	D-Mannitol (1-^{13}C, 98%)
CLM-4416	D-Mannitol (2-^{13}C, 99%)
CLM-6733	D-Mannitol (U-$^{13}C_6$, 99%)
CLM-358	D-Mannose (1-^{13}C, 99%)
CLM-1523	D-Mannose (2-^{13}C, 99%)
CLM-1192	D-Mannose (6-^{13}C, 99%)
CLM-6567	D-Mannose (U-$^{13}C_6$, 99%)
DLM-1193	D-Mannose (1-D, 98%)
DLM-1194	D-Mannose (2-D, 98%)
DLM-1195	D-Mannose (6,6-D_2, 98%)
CLM-1218	L-Mannose (1-^{13}C, 99%)
CLM-1196	D-Ribitol (1-^{13}C, 99%)

(continued)

Carbohydrates

Catalog No.	Description
CLM-768	D-Ribose (1-^{13}C, 99%)
CLM-1069	D-Ribose (2-^{13}C, 99%)
CLM-1066	D-Ribose (5-^{13}C, 99%)
CLM-4602	D-Ribose (1,2-$^{13}C_2$, 99%)
CLM-4830	D-Ribose (2,3,4,5-$^{13}C_4$, 99%)
CLM-3652	D-Ribose (U-$^{13}C_5$, 98%)
DLM-1070	D-Ribose (1-D, 98%)
DLM-1197	D-Ribose (2-D, 98%)
DLM-6559	D-Ribose (3-D, 98%)
DLM-7778	D-Ribose (5,5-D_2, 98%)
DLM-346	D-Ribose (2,3,4,5,5′-D_5, 98%)
CLM-8780	Sodium D-gluconate (1-^{13}C, 99%)
CLM-8781	Sodium D-gluconate (U-$^{13}C_6$, 99%)
CLM-1565	D-Sorbitol (1-^{13}C, 99%) (monohydrate or semihydrate)
CLM-8529	D-Sorbitol (U-$^{13}C_6$, 98%+)
CLM-8091	D-Sucrose (glucose-$^{13}C_6$, 98%)
CLM-7757	D-Sucrose ($^{13}C_{12}$, 98%)
CLM-1203	D-Talitol (1-^{13}C, 99%)

Catalog No.	Description
CLM-773	D-Talose (1-^{13}C, 99%)
CLM-1204	D-Talose (2-^{13}C, 99%)
CLM-1139	D-Threose (1-^{13}C, 99%) 1.8% in H_2O
CLM-1207	D-Threose (2-^{13}C, 99%) 1.8% in H_2O
CLM-1295	D-Xylitol (1-^{13}C, 99%)
CLM-1214	D-Xylitol (5-^{13}C, 99%)
CLM-7608	D-Xylitol (U-$^{13}C_5$, 99%)
CLM-1140	D-Xylose (1-^{13}C, 99%)
CLM-1524	D-Xylose (2-^{13}C, 99%)
CLM-8593	D-Xylose (3-^{13}C, 99%)
CLM-9083	D-Xylose (4-^{13}C, 99%)
CLM-1219	D-Xylose (5-^{13}C, 99%)
CLM-2456	D-Xylose (1,2-$^{13}C_2$, 99%)
CLM-6140	D-Xylose (U-$^{13}C_5$, 99%)
DLM-1215	D-Xylose (1-D, 99%)
DLM-1216	D-Xylose (2-D, 98%)
DLM-7121	D-Xylose (D_6, 98%)

Cell Growth Media

Yeast Media and Reagents

Catalog No.	Description
NLM-467	Ammonium chloride (^{15}N, 99%)
NLM-713	Ammonium sulfate ($^{15}N_2$, 99%)
CLM-1396	D-Glucose (U-$^{13}C_6$, 99%)
DLM-2062	D-Glucose (1,2,3,4,5,6,6-D_7, 97-98%)
CDLM-3813	D-Glucose (U-$^{13}C_6$, 99%; 1,2,3,4,5,6,6-D_7, 97-98%)
CLM-1510	Glycerol ($^{13}C_3$, 99%)
DLM-558	Glycerol (D_8, 99%)
CDLM-7745	Glycerol ($^{13}C_3$, 99%; D_8, 98%) CP 95%
CLM-359	Methanol (^{13}C, 99%)
CDLM-1035	Methanol (^{13}C, 99%; D_3, 98%)

Insect Cell Media

Catalog No.	Description
CGM-2000-N	BioExpress® 2000 (U-^{15}N, 98%)
CGM-2000-N-S	BioExpress® 2000 (U-^{15}N, 98%) (200 mL media kit)
CGM-2000-CN	BioExpress® 2000 (U-^{13}C, 98%; U-^{15}N, 98%)
CGM-2000-U	BioExpress® 2000 (unlabeled)
CGM-2000-U-S	BioExpress® 2000 (unlabeled) (200mL media kit)
CGM-2000-CUSTOM	BioExpress® 2000 (Labeled amino acids to be specified by customer at time of request)

Mammalian Cell Media

Catalog No.	Description
CGM-6000-N	BioExpress® 6000 (U-^{15}N, 98%)
CGM-6000-N-S	BioExpress® 6000 (U-^{15}N, 98%) (200 mL media kit)
CGM-6000-CN	BioExpress® 6000 (U-^{13}C, 98%; U-^{15}N, 98%)
CGM-6000-U-S	BioExpress® 6000 (unlabeled) (200 mL media kit)
CGM-6000-CUSTOM	BioExpress® 6000 (Labeled amino acids to be specified by customer at time of request)

BioExpress® is a registered trademark of Cambridge Isotope Laboratories, Inc.

CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

Cell Growth Media

Bacterial Media

BioExpress® 1000

Catalog No.	Description
CGM-1000-C	BioExpress® 1000 (U-^{13}C, 98%) (10x concentrate)
CGM-1000-D	BioExpress® 1000 (U-D, 98%) (10x concentrate)
CGM-1000-N	BioExpress® 1000 (U-^{15}N, 98%) (10x concentrate)
CGM-1000-CD	BioExpress® 1000 (U-^{13}C, 98%; U-D, 98%) (10x concentrate)
CGM-1000-CN	BioExpress® 1000 (U-^{13}C, 98%; U-^{15}N, 98%) (10x concentrate)
CGM-1000-DN	BioExpress® 1000 (U-D, 98%; U-^{15}N, 98%) (10x concentrate)
CGM-1000-CDN	BioExpress® 1000 (U-^{13}C, 98%; U-^{15}N, 98%; U-D, 98%) (10x concentrate)
CGM-1000-U	BioExpress® 1000 (unlabeled) (10x concentrate)

Celtone® Complete

Catalog No.	Description
CGM-1040-C	Celtone® Complete Medium (^{13}C, 98%+)
CGM-1040-D	Celtone® Complete Medium (D, 97%+)
CGM-1040-N	Celtone® Complete Medium (^{15}N, 98%+)
CGM-1040-CN	Celtone® Complete Medium (^{13}C, 98%+; ^{15}N, 98%+)
CGM-1040-DN	Celtone® Complete Medium (D, 97%+; ^{15}N, 98%+)
CGM-1040-CDN	Celtone® Complete Medium (^{13}C, 98%+; D, 97%+; ^{15}N, 98%+)
CGM-1040-U	Celtone® Complete Medium (unlabeled)

Celtone® Powder

Catalog No.	Description
CGM-1030P-C	Celtone® Base Powder (^{13}C, 98%+)
CGM-1030P-D	Celtone® Base Powder (D, 97%+)
CGM-1030P-N	Celtone® Base Powder (^{15}N, 98%+)
CGM-1030P-CN	Celtone® Base Powder (^{13}C, 98%+; ^{15}N, 98%+)
CGM-1030P-DN	Celtone® Base Powder (D, 97%+; ^{15}N, 98%+)
CGM-1030P-CDN	Celtone® Base Powder (^{13}C, 98%+; D, 97%+; ^{15}N, 98%+)
CGM-1030P-U	Celtone® Base Powder (unlabeled)

Spectra 9 Media

Catalog No.	Description
CGM-3030-C	Spectra 9 (^{13}C, 98%)
CGM-3030-D	Spectra 9 (D, 97%+)
CGM-3030-N	Spectra 9 (^{15}N, 98%)
CGM-3030-CN	Spectra 9 (^{13}C, 98%; ^{15}N, 98%)
CGM-3030-DN	Spectra 9 (D, 97%+; ^{15}N, 98%+)
CGM-3030-CDN	Spectra 9 (^{13}C, 98%; D, 97%+; ^{15}N, 98%)
CGM-3030-U	Spectra 9 (unlabeled)

Minimal Media Reagents

Catalog No.	Description
NLM-467	Ammonium chloride (^{15}N, 99%)
NLM-713	Ammonium sulfate ($^{15}N_2$, 99%)
DLM-4-99	Deuterium oxide (D, 99%)
DLM-4-99.8	Deuterium oxide (D, 99.8%)
DLM-4	Deuterium oxide (D, 99.9%)
CLM-1396	D-Glucose (U-$^{13}C_6$, 99%)
DLM-2062	D-Glucose (1,2,3,4,5,6,6-D_7, 97-98%)
CDLM-3813	D-Glucose (U-$^{13}C_6$, 99%; 1,2,3,4,5,6,6-D_7, 97-98%)
CLM-1510	Glycerol ($^{13}C_3$, 99%)
DLM-558	Glycerol (D_8, 99%)

See CIL Application Note 12 at www.isotope.com.

Cell-Free Protein Expression

Amino Acid Mixes for Cell-Free Protein Expression

Catalog No.	Description
CLM-1548	Algal amino acid mixture (16AA) (U-^{13}C, 97-99%)
DLM-2082	Algal amino acid mixture (16AA) (U-D, 98%)
NLM-2161	Algal amino acid mixture (16AA) (U-^{15}N, 98%)
CNLM-452	Algal amino acid mixture (16AA) (U-^{13}C, 97-99%; U-^{15}N, 97-99%)
DNLM-819	Algal amino acid mixture (16AA) (U-D, 98%; U-^{15}N, 98%)
CDNLM-2496	Algal amino acid mixture (16AA) (U-^{13}C, 97-99%; U-D, 97-99%; U-^{15}N, 97-99%)
ULM-2314	Algal amino acid mixture (16AA) (unlabeled)

Catalog No.	Description
DLM-6819	"Cell Free" amino acid mix (20AA) (U-D, 98%)
NLM-6695	"Cell Free" amino acid mix (20AA) (U-^{15}N, 96-98%)
CNLM-6696	"Cell Free" amino acid mix (20AA) (U-^{13}C, 97-99%+; U-^{15}N, 97-99%)
DNLM-6818	"Cell Free" amino acid mix (20AA) (U-D, 98%; U-^{15}N, 98%)
CDNLM-6784	"Cell Free" amino acid mix (20AA) (U-^{13}C, 97-99%; U-^{15}N, 97-99%; U-D, 97-99%)
ULM-7891	"Cell Free" amino acid mix (20AA) (unlabeled)

CFS Product Listing

Standard Reagents and Kits

Catalog No.	Short Description	Description
Transcription Reagents		
CFS-TSC-5TB	5x Transcription Buffer	Transcription buffer for WEPRO1240/2240 series
CFS-TSC-ENZ	SP6 RNA Polymerase + RNase Inhibitor	Enzymes for mRNA synthesis and RNase activity inhibition
CFS-TSC-NTP	NTP Mix	Mixture of high quality substrates for transcription
Translation Reagents		
CFS-WGE-1240	WEPRO1240	WGE (240 OD) for general purpose
CFS-WGE-1240G	WEPRO1240G	WGE (240 OD) to produce high purity GST-tagged proteins
CFS-WGE-1240H	WEPRO1240H	WGE (240 OD) to produce high purity His-tagged proteins
CFS-WGE-2240	WEPRO2240	WGE (240 OD) to produce labeled proteins; WEPRO1240 less amino acids
CFS-WGE-2240H	WEPRO2240H	WGE (240 OD) to produce His-tagged, labeled proteins; WEPRO1240H less amino acids
CFS-SUB-AMX	SUB-AMIX	Buffer containing ATP, GTP, and 20 amino acids; used with WEPRO1240 series
CFS-SUB-NAA	SUB-AMIX NA	Buffer containing no amino acids; used with WEPRO2240 series
CFS-SUB-AMX-N	SUB-AMIX (^{15}N, 97-99%)	Buffer containing ^{15}N-labeled amino acids; used with WEPRO2240 series
CFS-SUB-AMX-CN	SUB-AMIX (^{13}C, 97-99%; ^{15}N, 97-99%)	Buffer containing ^{13}C,^{15}N-labeled amino acids; used with WEPRO2240 series
CFS-SUB-AMX-CDN	SUB-AMIX (^{13}C, 97-99%; D, 97-99%; ^{15}N, 97-99%)	Buffer containing ^{13}C,D,^{15}N-labeled amino acids; used with WEPRO2240 series
Small Scale Expression Plus Kits		
CFS-EDX-PLUS	Premium Expression Kit	Trial expression kit using plasmid or for template for eight (8) 226-µl reactions
Large Scale Expression Kits		
CFS-TRI-1240	WEPRO1240 Expression Kit	Kit containing 2 mL WEPRO1240
CFS-TRI-1240G	WEPRO1240G Expression Kit	Kit containing 2 mL WEPRO1240G
CFS-TRI-1240H	WEPRO1240H Expression Kit	Kit containing 2 mL WEPRO1240H
CFS-TRI-2240H	WEPRO2240H Expression Kit	Kit containing 2 mL WEPRO2240H and SUB-AMIX NA
CFS-TRI-2240-N	WEPRO2240 (^{15}N) Expression Kit	Kit containing 2 mL WEPRO2240 and ^{15}N-labeled amino acids
CFS-TRI-2240-CN	WEPRO2240 (^{13}C,^{15}N) Expression Kit	Kit containing 2 mL WEPRO2240 and ^{13}C,^{15}N-labeled amino acids
CFS-TRI-2240-CDN	WEPRO2240 (^{13}C,D,^{15}N) Expression Kit	Kit containing 2 mL WEPRO2240 and ^{13}C,D,^{15}N-labeled amino acids

ENDEXT® is a registered trademark of CellFree Sciences.
Protemist® is a registered trademark of Emerald BioSystems.

CIL is a distributor of the above-referenced CFS products in the US and Europe.

Cell-Free Protein Expression

Catalog No	Short Description	Description
CFS-TRI-2240H-N	WEPRO2240H (^{15}N) Expression Kit	Kit containing 2 mL WEPRO2240H and ^{15}N-labeled amino acids
CFS-TRI-2240H-CN	WEPRO2240H (^{13}C, ^{15}N) Expression Kit	Kit containing 2 mL WEPRO2240H and ^{13}C,^{15}N-labeled amino acids
CFS-TRI-2240H-CDN	WEPRO2240H (^{13}C, D, ^{15}N) Expression Kit	Kit containing 2 mL WEPRO2240H and ^{13}C,D,^{15}N-labeled amino acids

High Performance Reagents and Kits

Transcription Reagents		
CFS-TSC-5TB-LM	5x Transcription Buffer LM	Transcription buffer for WEPRO7240/8240 series
CFS-TSC-ENZ	SP6 RNA Polymerase + RNase Inhibitor	Enzymes for mRNA synthesis and RNase activity inhibition
CFS-TSC-NTP	NTP Mix	Mixture of high quality substrates for transcription
Translation Reagents		
CFS-WGE-7240	WEPRO7240	High yield WGE (240 OD) for general purpose
CFS-WGE-7240G	WEPRO7240G	High yield WGE (240 OD) to produce high purity GST-tagged proteins
CFS-WGE-7240H	WEPRO7240H	High yield WGE (240 OD) to produce high purity His-tagged proteins
CFS-WGE-8240	WEPRO8240	High yield WGE (240 OD) to produce labeled proteins; WEPRO7240 less amino acids
CFS-WGE-8240H	WEPRO8240H	High yield WGE (240 OD) to produce high purity His-tagged, labeled proteins; WEPRO7240H less amino acids
CFS-SUB-SGC	SUB-AMIX SGC	Buffer for BL for general purpose; used with WEPRO7240 series
CFS-SUB-SGC-NAA	SUB-AMIX SGC NA	Buffer for BL, containing no amino acids; used with WEPRO8240 series
CFS-SUB-SG	SUB-AMIX SG	Buffer for FF for general purpose; used with WEPRO7240 series
CFS-SUB-SG-NAA	SUB-AMIX SG NA	Buffer for FF, containing no amino acids ; used with WEPRO8240 series
CFS-SUB-SGC-N	SUB-AMIX SGC (^{15}N, 97-99%)	Buffer for BL, containing ^{15}N-labeled amino acids; used with WEPRO8240 series
CFS-SUB-SGC-CN	SUB-AMIX SGC (^{13}C, 97-99%; ^{15}N, 97-99%)	Buffer for BL, containing ^{13}C,^{15}N-labeled amino acids; used with WEPRO8240 series
CFS-SUB-SGC-CDN	SUB-AMIX SGC (^{13}C, 97-99%; D, 97-99%; ^{15}N, 97-99%)	Buffer for BL, containing ^{13}C,D,^{15}N-labeled amino acids; used with WEPRO8240 series
CFS-SUB-SG-N	SUB-AMIX SG (^{15}N, 97-99%)	Buffer for FF, containing ^{15}N-labeled amino acids; used with WEPRO8240 series
CFS-SUB-SG-CN	SUB-AMIX SG (^{13}C, 97-99%; ^{15}N, 97-99%)	Buffer for FF, containing ^{13}C,^{15}N-labeled amino acids; used with WEPRO8240 series
CFS-SUB-SG-CDN	SUB-AMIX SG (^{13}C, 97-99%; D, 97-99%; ^{15}N, 97-99%)	Buffer for FF, containing ^{13}C,D,^{15}N-labeled amino acids; used with WEPRO8240 series
Large Scale Expression Kits		
CFS-TRI-7240	WEPRO7240 Expression Kit	Contains 2mL WEPRO7240
CFS-TRI-7240G	WEPRO7240G Expression Kit	Contains 2 mL WEPRO7240G
CFS-TRI-7240H	WEPRO7240H Expression Kit	Contains 2 mL WEPRO7240H
CFS-TRI-8240H-N	WEPRO8240H (^{15}N) Expression Kit	Contains 2 mL WEPRO8240H and ^{15}N-labeled amino acids
CFS-TRI-8240H-CN	WEPRO8240H (^{13}C,^{15}N) Expression Kit	Contains 2 mL WEPRO8240H and ^{13}C,^{15}N-labeled amino acids
CFS-TRI-8240H-CDN	WEPRO8240H (^{13}C,D,^{15}N) Expression Kit	Contains 2 mL WEPRO8240H and ^{13}C,D,^{15}N-labeled amino acids

WGE: Wheat germ extract BL: Bilayer method FF: Filter-and-Feed method

Chemical Tagging Reagents and Related Products

Catalog No.	Description
CLM-173	Acetaldehyde (1,2-$^{13}C_2$, 99%)
DLM-112	Acetaldehyde (D_4, 99%)
CLM-1159	Acetic anhydride (1,1′-$^{13}C_2$, 99%)
CLM-1160	Acetic anhydride (2,2′-$^{13}C_2$, 99%)
CLM-1161	Acetic anhydride (1,1′,2,2′-$^{13}C_4$, 99%)
DLM-1162	Acetic anhydride (D_6, 98%)
CDLM-9271	Acetic anhydride ($^{13}C_4$, 99%; D_6, 98%)
DLM-9	Acetone (D_6, 99.9%)
CLM-1260	Acetonitrile (1-^{13}C, 99%)
CLM-704	Acetyl chloride (1,2-$^{13}C_2$, 99%)
DLM-247	Acetyl chloride (D_3, 98%)
CDLM-6208	Acetyl chloride ($^{13}C_2$, 99%; D_3, 98%)
CLM-9270	Acrylamide (1-^{13}C, 99%)
CLM-813	Acrylamide (1,2,3-$^{13}C_3$, 99%)
DLM-821	Acrylamide (2,3,3-D_3, 98%)
OLM-7858	Adenosine 5′-triphosphate, sodium salt (γ-$^{18}O_4$, 94%+)
CLM-8906	*S*-Adenosyl-L-homocysteine (adenosine-$^{13}C_{10}$, 98%)
CLM-8755	β-Alanine (3-^{13}C, 99%)
CLM-8756	β-Alanine (1,2,3-$^{13}C_3$, 99%)
CNLM-3440	β-Alanine (3-^{13}C, 99%; ^{15}N, 98%)
CNLM-3946	β-Alanine (U-$^{13}C_3$, 98%+; ^{15}N, 96-99%)
CLM-714	Aniline ($^{13}C_6$, 99%)
CLM-466	Barium carbonate (^{13}C, 98%+)
CLM-182	Benzene ($^{13}C_6$, 99%)
CLM-1813	Benzoic acid (ring-$^{13}C_6$, 99%)
CLM-3010	Benzoyl chloride (carbonyl-^{13}C, 99%)
DLM-595	Benzoyl chloride (D_5, 99%)
CLM-1339	Bromoacetic acid (1,2-$^{13}C_2$, 99%)
CLM-871	Bromobenzene ($^{13}C_6$, 99%)
DLM-398	Bromobenzene (D_5, 99%)
DLM-103	2-Bromoethanol (1,1,2,2-D_4, 98%) CP ≥95%
CLM-1829	Chlorobenzene ($^{13}C_6$, 99%)
DLM-341	1,4-Dibromobenzene (D_4, 98%)
CDLM-301	1,2-Dibromoethane (1,2-$^{13}C_2$, 99%; D_4, 98%)
CLM-495	Diethyl malonate (2-^{13}C, 99%)
CLM-3603	Diethyl malonate (1,2,3-$^{13}C_3$, 99%)
DLM-267	Dimethylamine (D_6, 99%) gas
CLM-266	Dimethyl sulfate ($^{13}C_2$, 99%)
DLM-196	Dimethyl sulfate (D_6, 98%)
DLM-2622	DL-1,4-Dithiothreitol (D_{10}, 98%)
DLM-6785	1,2-Ethanedithiol (1,1,2,2-D_4, 98%)
DLM-552	Ethanolamine (D_4, 98%)
CLM-3297	Ethyl acetoacetate (1,2,3,4-$^{13}C_4$, 99%)
CLM-1009	Ethyl bromoacetate (1-^{13}C, 99%)
CLM-1011	Ethyl bromoacetate (1,2-$^{13}C_2$, 99%)
DLM-271	Ethylene oxide (D_4, 98%) (stabilized with 0.1% hydroquinone)

Catalog No.	Description
DLM-6711	*N*-Ethylmaleimide (ethyl-D_5, 98%)
CLM-806	Formaldehyde (^{13}C, 99%) (~20% w/w in H_2O)
DLM-805	Formaldehyde (D_2, 98%) (~20% w/w in D_2O)
CDLM-4599	Formaldehyde (^{13}C, 99%; D_2, 98%) (20% w/w in D_2O)
DLM-1229	Glycerol (1,1,2,3,3-D_5, 99%)
CNLM-7138	Guanidine•HCl (^{13}C, 99%; $^{15}N_3$, 98%)
CNLM-7333	Guanidine•HBR (^{13}C, 99%: $^{15}N_3$, 98%)
DLM-7249	Iodoacetamide (D_4, 98%)
CLM-3264	Iodoacetic acid (2-^{13}C, 99%)
CLM-8824	Iodoacetic acid ($^{13}C_2$, 99%)
DLM-272	Iodoethane (D_5, 99%) + copper wire
DLM-1136	Isopropanol (dimethyl-D_6, 98%)
DLM-1981	Methanesulfonic acid (D_4, 97-98%)
DLM-598	Methanol (D_3, 99.5%)
CDLM-688	Methanol (^{13}C, 99%; D_4, 99%)
CDLM-8241	Methylamine•HCl (^{13}C, 99%; D_3, 98%)
CDNLM-8182	Methylamine•HCl (^{13}C, 99%; methyl-D_3, 98%; ^{15}N, 98%)
CNLM-6088	*O*-Methylisourea hydrogen chloride (isourea-^{13}C, 99%; $^{15}N_2$, 98%) CP ≥95%
DLM-2872	Nicotinic acid, ethyl ester (2,4,5,6-D_4, 98%)
CLM-675	Nitrobenzene ($^{13}C_6$, 99%)
CLM-6586	2-Nitrobenzenesulfenyl chloride ($^{13}C_6$, 99%)
CLM-3981	Octanoic acid ($^{13}C_8$, 99%)
CLM-216	Phenol ($^{13}C_6$, 99%)
DLM-7731	Phenyl isocyanate (phenyl-D_5, 98%)
OLM-1057	Phosphoric acid ($^{18}O_4$, 96%) (80-85% in ^{18}O water)
NLM-111	Potassium cyanide (^{15}N, 98%+)
OLM-7493	Potassium dihydrogen phosphate ($^{18}O_4$, 97%)
OLM-7523	Potassium phosphate (^{18}O, 97%)
DLM-599	Propionic acid (D_6, 98%)
DLM-3305	Propionic anhydride (D_{10}, 98%)
DLM-1067	1,2-Propylene oxide (D_6, 98%) (stabilized with hydroquinone)
DLM-3126	Sodium acetate (D_3, 99%)
CDLM-611	Sodium acetate (1-^{13}C, 99%; D_3, 98%)
CDLM-1240	Sodium acetate (2-^{13}C, 99%; D_3, 98%)
CDLM-3457	Sodium acetate (1,2-$^{13}C_2$, 99%; D_3, 98%)
DLM-226	Sodium borodeuteride (D_4, 99%) CP 95%+
DLM-7364	Sodium cyanoborodeuteride (D_3, 98%) CP 95%+
CLM-1571	Succinic acid ($^{13}C_4$, 99%)
CDLM-7754	Succinic acid ($^{13}C_4$, 99%; 2,2,3,3-D_4, 98%)
CLM-2473	Succinic anhydride (1,2,3,4-$^{13}C_4$, 99%)
DLM-833	Succinic anhydride (D_4, 98%)
DLM-6143	Suberic acid (2,2,7,7-D_4, 98%)
DLM-1176	Toluene (ring-D_5, 98%)
CLM-311	Urea (^{13}C, 99%)
NLM-233	Urea ($^{15}N_2$, 98%+)

Fatty Acids

Catalog No.	Description
DLM-2497	Algal fatty acid mixture (methyl esters) (U-D, 96-98%)
CLM-1239	Arachidic acid (1-^{13}C, 99%) (eicosanoic acid)
DLM-1234	Arachidic acid (methyl-D_3, 98%)
DLM-1233	Arachidic acid (D_{39}, 98%)
DLM-2006	Decanoic acid (methyl-D_3, 98%)
DLM-270	Decanoic acid (D_{19}, 98%)
DLM-7974	Dimyristin (dimyristoyl-D_{54}, 98%)
DLM-8273	Dipalmitin (D_{67}, 98%)
CLM-8388	Docosahexaenoic acid (U-$^{13}C_{22}$, 95%+) CP 95%+
CLM-8398	Docosahexaenoic acid, methyl ester (docosahexaenoate-$^{13}C_{22}$, 95%+) CP 90%+
CLM-8398-HP	Docosahexaenoic acid, methyl ester (docosahexaenoate-$^{13}C_{22}$, 95%+)
DLM-9180	Docosanoic acid (22,22,22-D_3, 98%)
CLM-8274	Ethyl hexanoate (hexanoate-$^{13}C_6$, 99%)
DLM-1308	Heptadecanoic acid (methyl-D_3, 98%)
DLM-6905	Heptadecanoic acid (D_{33}, 98%)
DLM-1820	Heptanoic acid (2,2,3,3-D_4, 98%)
DLM-2731	Heptanoic acid (D_{13}, 98%)
DLM-8510	Hexacosanoic acid (12,12,13,13-D_4, 98%)
DLM-2922	DL-3-Hydroxytetradecanoic acid (2,2,3,4,4-D_5, 96%)
DLM-3062	Lauric acid (methyl-D_3, 99%)
CLM-6855	Linoleic acid (U-$^{13}C_{18}$, 98%) 94%+ (<10% *cis*/*trans* isomer)
CLM-2119	Linoleic acid, ethyl ester (1-^{13}C, 99%)
CLM-3960	Linoleic acid, ethyl ester (linoleate-U-$^{13}C_{18}$, 98%) CP 95%+
DLM-1909	Linoleic acid, ethyl ester (9,10,12,13-D_4, 95%)
DLM-227	Linoleic acid, ethyl ester (17,17,18,18,18-D_5, 98%)
DLM-766	Linoleic acid, ethyl ester (D_{31}, 98%) CP 95%+
CLM-8395	Linoleic acid, methyl ester (linoleate-$^{13}C_{18}$, 98%+) CP 95%
CLM-6229	Linoleic acid, potassium salt (1-^{13}C, 99%)
CLM-8835	Linoleic acid, potassium salt (U-$^{13}C_{18}$, 98%) CP 97%
DLM-2160	Linolenic acid (D_{29}, 98%)
DLM-2351	Linolenic acid, ethyl ester (17,17,18,18,18-D_5, 98%) CP 90%
CLM-8455	Mixed fatty acids (U-^{13}C, 98%+)
DLM-8572	Mixed fatty acids (U-D, 98%)
CDLM-8376	Mixed fatty acids (U-^{13}C, 98%+; U-D, 97%+)
CLM-8381	Mixed fatty acids methyl esters (U-^{13}C, 98%+) (terminal ester unlabeled) CP 95%
DLM-2497	Mixed fatty acid methyl esters (U-D, 96-98%)
CLM-8373	Mixed triglycerides (U-^{13}C, 98%+)
DLM-8375	Mixed triglycerides (U-D, 97%+)
CLM-1844	Myristic acid (1-^{13}C, 99%)
CLM-3665	Myristic acid (1,2,3-$^{13}C_3$, 99%)
DLM-1039	Myristic acid (methyl-D_3, 98%)
DLM-208	Myristic acid (D_{27}, 98%)
CLM-6228	Myristic acid, potassium salt (1-^{13}C, 99%)
CLM-8724	Nonanoic acid (U-$^{13}C_9$, 98%)
DLM-4533	DL-α-Phosphatidylcholine, dimyristoyl (DMPC) (D_{72}, 98%)
DLM-605	L-α-Phosphatidylcholine, dimyristoyl (DMPC) (dimyristoyl-D_{54}, 98%) CP 95%
DLM-606	L-α-Phosphatidylcholine, dipalmitoyl (DPPC) (dipalmitoyl-D_{62}, 98%) CP 95%
DLM-7558	L-Phosphatidylglycerol, dimyristoyl (DMPG) (dimyristoyl-D_{54}, 98%)
DLM-7557	L-Phosphatidylglycerol, dipalmitoyl (DPPG) (dipalmitoyl-D_{62}, 98%)
CLM-293	Octanoic acid (1-^{13}C, 99%)
CLM-3827	Octanoic acid (1,2-$^{13}C_2$, 99%)
CLM-2721	Octanoic acid (1,2,3,4-$^{13}C_4$, 99%)
CLM-3981	Octanoic acid ($^{13}C_8$, 99%)
DLM-619	Octanoic acid (D_{15}, 98%)
CLM-4259	Octanoyl-1,3-diolein (octanoyl-1,2-$^{13}C_2$, 99%)
CLM-3707	2-Octanoyl-1,3-distearin (octanoic-1-^{13}C, 99%)
CLM-4258	2-Octanoyl-1,3-distearin (octanoyl-1,2-$^{13}C_2$, 99%)
CLM-149	Oleic acid (1-^{13}C, 99%)
CLM-2492	Oleic acid (methyl-^{13}C, 99%)
CLM-460	Oleic acid (U-$^{13}C_{18}$, 98%) CP 95%
DLM-689	Oleic acid (9,10-D_2, 97%)
DLM-2968	Oleic acid (11,11-D_2, 98%)
DLM-1891	Oleic acid (D_{33}, 98%)
CLM-3959	Oleic acid, ethyl ester (oleate-U-$^{13}C_{18}$, 98%+)
DLM-8747	Oleic acid, ethyl ester (D_{33}, 98%) CP 95%+
CLM-4337	Oleic acid, methyl ester (oleate-$^{13}C_{18}$, 98%+)
CLM-4477	Oleic acid, potassium salt (1-^{13}C, 99%)
CLM-8856	Oleic acid, potassium salt (U-$^{13}C_{18}$, 98%) CP 95%
DLM-8837	Oleic acid, potassium salt (15,15,16,16,17,17,18,18,18-D_9, 98%)
CLM-6230	Oleic acid, sodium salt (1-^{13}C, 99%)
CLM-8763	Oleic acid, sodium salt (U-$^{13}C_{18}$, 98%)
CLM-150	Palmitic acid (1-^{13}C, 99%)
CLM-2120	Palmitic acid (2-^{13}C, 99%)
CLM-2253	Palmitic acid (methyl-^{13}C, 99%)
CLM-214	Palmitic acid (1,2-$^{13}C_2$, 99%)
CLM-7896	Palmitic acid (1,2,3,4-$^{13}C_4$, 99%)
CLM-409	Palmitic acid (U-$^{13}C_{16}$, 98%)
DLM-8673	Palmitic acid (12-D_1, 98%)
DLM-1153	Palmitic acid (2,2-D_2, 98%)
DLM-2889	Palmitic acid (3,3-D_2, 98%)
DLM-2890	Palmitic acid (9,9-D_2, 98%)
DLM-2891	Palmitic acid (13,13-D_2, 98%)
DLM-611	Palmitic acid (methyl-D_3, 98%)
DLM-2892	Palmitic acid (5,5,6,6-D_4, 98%)
DLM-2893	Palmitic acid (7,7,8,8-D_4, 98%)
DLM-2894	Palmitic acid (11,11,12,12-D_4, 98%)
DLM-2895	Palmitic acid (9,9,...16,16,16-D_{17}, 98%) CP 97%
DLM-215	Palmitic acid (D_{31}, 98%)
CLM-3957	Palmitic acid, ethyl ester (palmitate-U-$^{13}C_{16}$, 98%+) CP 95%
DLM-8793	Palmitic acid, ethyl ester (D_{31}, 98%)
CLM-8390	Palmitic acid, methyl ester (palmitate-$^{13}C_{16}$, 98%+)
CLM-2241	Palmitoleic acid ($^{13}C_{16}$, 99%) CP 95-99%
CLM-3958	Palmitoleic acid, ethyl ester (palmitoleate-U-$^{13}C_{16}$, 98%+) CP 95%+
CLM-8391	Palmitoleic acid, methyl ester (palmitoleate-$^{13}C_{16}$, 98%+)
CLM-8384	Palmitolenic acid (U-$^{13}C_{16}$, 98%+) CP >90%
CLM-8392	Palmitolenic acid, methyl ester (palmitolenate-$^{13}C_{16}$, 98%+)
DLM-1307	Pentadecanoic acid (methyl-D_3, 98%)

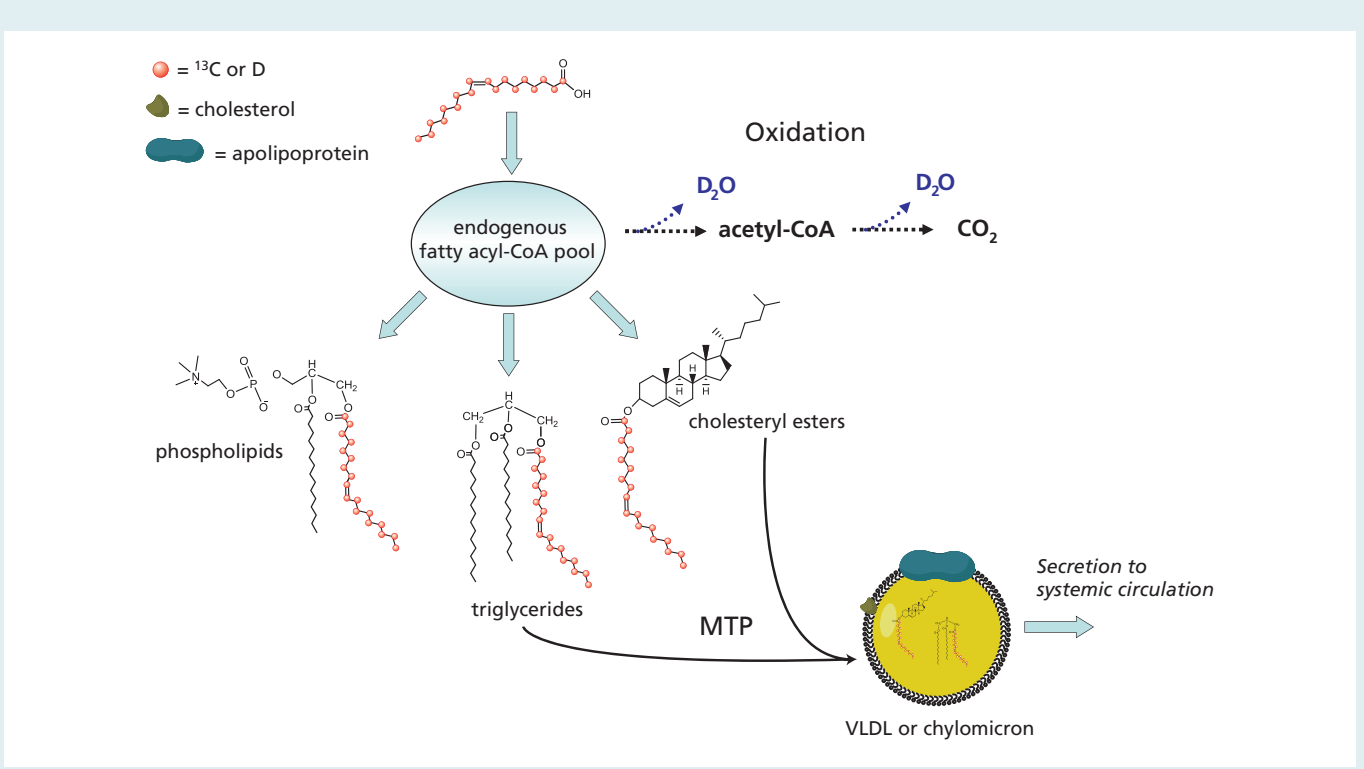
CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

(continued)

Fatty Acids

Catalog No.	Description
DLM-572	Pentanoic acid (D_9, 98%)
CLM-1889	Potassium palmitate (1-^{13}C, 99%)
CLM-6865	Potassium palmitate (1,2,3,4-$^{13}C_4$, 99%)
CLM-3943	Potassium palmitate (U-$^{13}C_{16}$, 98%+)
DLM-3773	Potassium palmitate (2,2-D_2, 97%)
DLM-6199	Potassium palmitate (methyl-D_3, 98%)
DLM-6033	Potassium palmitate (7,7,8,8-D_4, 98%)
CLM-1948	Sodium octanoate (1-^{13}C, 99%)
CLM-3876	Sodium octanoate (1,2,3,4-$^{13}C_4$, 99%)
CLM-3980	Sodium octanoate (2,4,6,8-$^{13}C_4$, 99%)
CLM-174	Sodium palmitate (1-^{13}C, 99%)
CLM-6059	Sodium palmitate ($^{13}C_{16}$, 98%+)
CLM-490	Stearic acid (methyl-^{13}C, 99%)
CLM-676	Stearic acid (1-^{13}C, 99%)
CLM-6990	Stearic acid (U-$^{13}C_{18}$, 98%) CP 97%

Catalog No.	Description
DLM-1154	Stearic acid (methyl-D_3, 98%)
DLM-379	Stearic acid (D_{35}, 98%)
CLM-8731	Stearic acid, ethyl ester (stearate-$^{13}C_{18}$, 98%+)
DLM-8748	Stearic acid, ethyl ester (D_{35}, 98%)
CLM-8394	Stearic acid, methyl ester (stearate-$^{13}C_{18}$, 98%+)
CLM-6227	Stearic acid, potassium salt (1-^{13}C, 99%)
DLM-7302	Tetracosanoic acid (D_{47}, 98%)
DLM-9179	Tetracosanoic acid (9,9,10,10-D_4, 98%)
DLM-7487	Tetradecanoic acid (13,13,14,14,14-D_5, 98%)
DLM-1392	Tridecanoic acid (D_{25}, 98%)
CLM-162	Trioctanoin (1,1,1-$^{13}C_3$, 99%)
CLM-163	Triolein (1,1,1-$^{13}C_3$, 99%)
CLM-164	Tripalmitin (1,1,1-$^{13}C_3$, 99%)
CLM-8445	Tripalmitin (glyceryl-$^{13}C_3$, 99%)
DLM-9044	Tripalmitin (D_{98}, 98%)
DLM-7875	Tristearin (tristearoyl-D_{105}, 98%)



Schematic illustration showing the incorporation of [^{13}C] or [D] oleic acid into larger lipids.

For further information on fatty acid and lipid metabolism, please see the application note beginning on page 74.

CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

Gases

Catalog No.	Description
DLM-389	Ammonia (D_3, 99%)
NLM-107	Ammonia (^{15}N, 98%)
NLM-107-10	Ammonia (^{15}N, 10%)
NLM-859	Ammonia (^{14}N, 99.99%)
NDLM-860	Ammonia (^{15}N, 98%+; D_3, 98%)
ARLM-6672	Argon (^{36}Ar, 99.5%)
CLM-1217	Bromomethane (^{13}C, 99%)
DLM-401	Bromomethane (D_3, 99%)
CLM-7114	1,3-Butadiene ($^{13}C_4$, 99%) + hydroquinone
DLM-877	1,3-Butadiene (1,1,4,4-D_4, 98%) + hydroquinone
DLM-876	1,3-Butadiene (D_6, 98%) + hydroquinone
CLM-8994	*n*-Butane (1-^{13}C, 99%)
DLM-1610	*n*-Butane (D_{10}, 98%)
CLM-3505	1-Butene (1-^{13}C, 99%)
CLM-4309	1-Butene (1,2-$^{13}C_2$, 99%)
DLM-4623	1-Butene (4,4,4-D_3, 98%)
DLM-2033	1-Butene (D_8, 98%)
CLM-185-10	Carbon dioxide (^{13}C, 10%)
CLM-185	Carbon dioxide (^{13}C, 99%) (<1% ^{18}O)
CLM-3781	5% CO_2 in air baseline calibrant gas with delta value = -22.71 *vs.* PDB
CLM-3782	5% CO_2 in air mid-level calibrant gas at 7.21 delta units above baseline calibrant gas
CLM-3783	5% CO_2 in air high-level calibrant gas at 13.02 delta units above baseline calibrant gas
CLM-3785	5% CO_2 in air low-level calibrant gas at 13.32 delta units above baseline calibrant gas
CLM-9026	5% CO_2 in air mid-level calibrant gas at 9.11 delta units above baseline calibrant gas
CLM-880	Carbon dioxide (^{12}C, 99.95%)
CLM-477	Carbon dioxide (^{12}C, 99.99%)
CLM-709	Carbon dioxide (^{12}C, 99.999%)
OLM-186	Carbon dioxide ($^{18}O_2$, 95%)
COLM-801	Carbon dioxide (^{13}C, 99%; $^{17}O_2$, 10%)
COLM-802	Carbon dioxide (^{13}C, 99%; $^{18}O_2$, 90%)
COLM-881	Carbon dioxide (^{12}C, 99.95%; $^{17}O_2$, 10%)
COLM-882	Carbon dioxide (^{12}C, 99.95%; $^{18}O_2$, 95%)
COLM-1313	Carbon dioxide (^{12}C, 99.95%; $^{18}O_2$, 50%)
CLM-189	Carbon monoxide (^{13}C, 99%) (<2% ^{18}O)
CLM-837	Carbon monoxide (^{12}C, 99.95%)
CLM-424	Carbon monoxide (^{12}C, 99.99%)
CLM-425	Carbon monoxide (^{12}C, 99.999%)
OLM-190	Carbon monoxide (^{18}O, 95%)
COLM-885	Carbon monoxide (^{13}C, 99%; ^{18}O, 95%)
CLM-4619	Carbonyl sulfide (^{13}C, 99%)
CLM-6582	Chloroethane (random-^{13}C, 99%)
CLM-3259	Chloroethane (2-^{13}C, 99%)
DLM-2809	Chloroethane (2-D, 98%)
DLM-1612	Chloroethane (2,2,2-D_3, 98%)
DLM-1171	Chloroethane (D_5, 98%)
CGM-P39	Cyclotron target gas mixture (code I-460) 39 ($^{15}N_2$, 99%):1 O_2 CP 99.99%

Catalog No.	Description
DLM-408	Deuterium (D, 99.8%) (D_2, 99.6% +HD, 0.4%)
DLM-1329	Deuterium (D, 99.9%) (D_2, 99.8% +HD, 0.2%)
DLM-1003	Deuterium (D, 99.96%) <400 ppm HD
DLM-342	Deuterium bromide (D, 99%)
DLM-458	Deuterium chloride (D, 99%)
DLM-194	Deuterium hydride (D, 97%)
DLM-596	Deuterium iodide (D, 98%)
DLM-791	Deuterium sulfide (D_2, 98%)
DLM-1558	Dichlorofluoromethane (D, 98%) CP 96%
CLM-1604	Difluoromethane (^{13}C, 99%)
DLM-1614	Difluoromethane (D_2, 98%)
CDLM-6057	Difluoromethane (^{13}C, 99%; D_2, 98%)
DLM-267	Dimethylamine (D_6, 99%)
DLM-506	Dimethylamine (D_7, 99%)
NLM-2304	Dimethylamine (^{15}N, 98%+)
CDLM-7280	Dimethylamine•HCl ($^{13}C_2$, 99%; D_6, 98%)
CLM-1517	Dimethyl ether ($^{13}C_2$, 99%)
DLM-340	Dimethyl ether (D_6, 99%)
CLM-2187	Ethane (1-^{13}C, 99%)
DLM-276	Ethane (D_6, 98%)
DLM-3857	Ethylamine (D_5, 98%)
CLM-415	Ethylene (1,2-$^{13}C_2$, 99%)
DLM-6610	Ethylene (D_1, 98%)
DLM-2446	Ethylene (1,1-D_2, 98%)
DLM-416	Ethylene (1,2-D_2, 98%) *cis*/*trans* mix
DLM-1530	Ethylene (*cis*-1,2-D_2, 98%)
DLM-740	Ethylene (*trans*-1,2-D_2, 98%)
DLM-347	Ethylene (D_4, 98%)
CDLM-6684	Ethylene ($^{13}C_1$, 99%; D_4, 98%)
CDLM-8024	Ethylene ($^{13}C_2$, 99%; D_4, 98%)
CLM-1014	Fluoromethane (^{13}C, 99%)
DLM-1015	Fluoromethane (D_3, 99%)
DLM-1809	Isobutane (D_{10}, 98%)
KRLM-4656	Krypton-78 (^{78}Kr, 99%)
KRLM-6794	Krypton-82 (^{82}Kr, 99%)
KRLM-7397	Krypton-86 (^{86}Kr)
CLM-1033	Methane (^{12}C, 99.95%)
CLM-427	Methane (^{12}C, 99.99%)
CLM-392	Methane (^{12}C, 99.999%)
CLM-429	Methane (^{13}C, 99%)
CLM-3590	Methane (^{13}C, 99.9%)
DLM-1257	Methane (D_1, 98%)
DLM-1343	Methane (D_2, 98%)
DLM-1344	Methane (D_3, 98%)
DLM-144	Methane (D_4, 99%)
CDLM-469	Methane (^{13}C, 99%; D_4, 99%)
CDLM-1616	Methane (^{12}C, 99.95%; D_4, 98%)
CLM-3961	Methanethiol (^{13}C, 99%)
DLM-2643	Methanethiol (D_3, 99%)

(continued)

Gases

Catalog No.	Description
CLM-1617	Methylamine (^{13}C, 99%)
DLM-1500	Methylamine (D_3, 98%)
DLM-1618	Methylamine (D_2, 98%)
DLM-1501	Methylamine (D_5, 98%)
NLM-1621	Methylamine (^{15}N, 98%+)
CNLM-302	Methylamine (^{13}C, 99%; ^{15}N, 99%)
CLM-7250	2-Methylpropene (2-^{13}C, 99%)
DLM-1623	Methyl vinyl ether (methyl-D_3, 98%)
DLM-1624	Methyl vinyl ether (vinyl-D_3, 98%)
NE-2099	Neon (^{20}Ne, 99.95%)
NE-2299	Neon (^{22}Ne, 99.9%)
NLM-823	Nitric oxide (^{15}N, 98%+)
NLM-363	Nitrogen ($^{15}N_2$, 98%+)
NLM-1619	Nitrogen dioxide (^{15}N, 99%)
NLM-1045	Nitrous oxide (1-^{15}N, 98%+)
NLM-1044	Nitrous oxide (2-^{15}N, 98%+)
NLM-1046	Nitrous oxide ($^{15}N_2$, 98%+)
OLM-212	Oxygen ($^{18}O_2$, 97%) CP >99.8%
OLM-213-70	Oxygen ($^{17}O_2$, 70%)
DLM-2703	Phosphine (D_3, 98%)
DLM-4732	Propadiene (D_4, 98%)
CLM-403	Propane (1-^{13}C, 99%)
CLM-2726	Propane (2-^{13}C, 99%)
DLM-3676	Propane (2,2-D_2, 98%)
DLM-3476	Propane (1,1,1,3,3,3-D_6, 98%)
DLM-153	Propane (D_8, 98%)

Catalog No.	Description
CLM-4254	Propene (1-^{13}C, 99%)
CLM-7210	Propene (2-^{13}C, 99%)
CLM-1514	Propene (3-^{13}C, 99%)
DLM-4624	Propene (1,1-D_2, 98%)
DLM-1626	Propene (3,3,3-D_3, 98%)
DLM-304	Propene (D_6, 98%)
CLM-6188	Propyne (1,2-$^{13}C_2$, 99%)
CLM-6189	Propyne (1,2,3-$^{13}C_3$, 99%)
DLM-4303	Propyne (1-D, 98%)
DLM-4302	Propyne (methyl-D_3, 98%)
DLM-4775	Propyne (D_4, 98%)
DLM-3823	Trimethylamine (dimethyl-D_6, 98%)
DLM-4638	Trimethylamine (D_8, 98%)
DLM-603	Trimethylamine (D_9, 98%)
DLM-3344	Vinyl bromide (D_3, 98%) + hydroquinone
CLM-472	Vinyl chloride ($^{13}C_2$, 99%) + hydroquinone
DLM-2390	Vinyl chloride (1-D_1, 98%)
XELM-428	Xenon (^{124}Xe, 99.9%)
XELM-430	Xenon (^{129}Xe, 99%)
XELM-8114	Xenon (^{131}Xe, 99%+)
XELM-7261	Xenon (^{132}Xe, 99.5%+)
XELM-8986	Xenon (^{134}Xe, 99%)

Gas Packaging

Catalog No.	Description
CODE U-0.3	0.3 L Aluminum cylinder with brass CGA 350 valve
CODE U-1	1 L Aluminum cylinder with brass CGA 350 valve
CODE U-2.9	2.9 L Aluminum cylinder with brass CGA 350 valve
CODE U-10.5	10.5 L Aluminum cylinder with brass CGA 350 valve
Breath Test	
BT-3L-KIT	Breath test adaptor set for use with CGA-580 cylinder
BTB-1300-20	Breath collection bag (aluminum lined) 1300 mL for baseline breath test
BTB-300-25	Breath test bags (aluminum lined) 300 mL for time course breath test sample
BT-LB-KIT	Breath test adaptor set for use with CGA-110/170 lecture bottle
Cylinder Valve	
CODE G-3	3 L carbon steel cylinder CGA 580 packless brass angle
CODE G-8	8 L carbon steel cylinder CGA 580 packless brass angle
CODE H-3	3 L carbon steel cylinder CGA 350 packless brass angle
CODE H-8	8 L carbon steel cylinder CGA 350 packless brass angle
CODE H-45	45 L carbon steel cylinder CGA 350 packless brass angle
CODE L	44 L carbon steel cylinder CGA 350 packless brass angle
CODE T-3	3 L carbon steel cylinder CGA 320 brass angle
CODE T-8	8 L carbon steel cylinder CGA 320 packless brass angle
CODE O	CGA 110 to 1/4" NPT brass adaptor
CODE P	CGA 110 to 1/4" NPT stainless steel adaptor
CODE R	CGA 170 control valve for code C lecture bottles
CODE S	CGA 180 to 1/8" NPT (m) adaptor stainless steel
CODE X	CGA-580 HPT 500 two-stage brass regulator for non-corrosive gases
CODE Y	CGA 170 regulator for code C lecture bottles for non-corrosive gases
CODE A	440 mL lecture bottle 1/4" NPT brass Nupro straight/male
CODE B	440 mL lecture bottle 1/4" NPT stainless steel Nupro straight/male

Catalog No.	Description
CODE C	440 mL lecture bottle CGA 110/170 brass angle
CODE D	440 mL lecture bottle CGA 110 stainless steel straight/female
CODE E	440 mL lecture bottle CGA 110 monel straight/female
CODE F	440 mL lecture bottle CGA 350 packless brass angle
CODE M	440 mL lecture bottle CGA 110/180 AL-SI-bronze valve angle
CODE N	440 mL lecture bottle CGA 580 packless brass angle
CODE Q	440 mL lecture bottle 1/4" NPT stainless steel Whitey straight/male
CODE I-75	75 mL stainless steel 1/4" NPT brass nupro straight/male
CODE I-150	150 mL stainless steel 1/4" NPT brass Nupro straight/male
CODE I-300	300 mL stainless steel 1/4" NPT brass Nupro straight/male
CODE I-460	460 mL stainless steel 1/4" NPT brass Nupro straight/male
CODE J-75	75 mL stainless steel 1/4" NPT stainless steel Nupro straight/male
CODE J-150	150 mL stainless steel 1/4" NPT stainless steel Nupro straight/male
CODE J-300	300 mL stainless steel 1/4" NPT stainless steel Nupro straight/male
CODE J-460	460 mL stainless steel 1/4" NPT stainless steel Nupro straight/male
CODE J-HP-460	460 mL stainless steel Swagelok-Nupro SS-DSM4F4A
CODE K-75	75 mL stainless steel 1/4" NPT stainless steel Whitey straight/male
CODE K-150	150 mL stainless steel 1/4" NPT stainless steel Whitey straight/male
CODE K-300	300 mL stainless steel 1/4" NPT stainless steel Whitey straight/male
CODE K-460	460 mL stainless steel 1/4" NPT stainless steel Whitey straight/male
CODE K-500	500 mL stainless steel 1/4" NPT DOT 3E1800
METAL BOX	Metal box required for select gas shipments via air

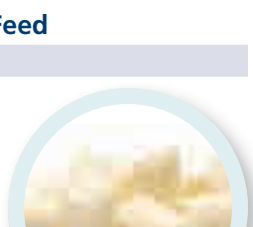
MouseExpress® Mouse Feed

MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed

Catalog No.	Description
MF-LYS-C	MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed
MF-LYS-C-IR	MouseExpress® L-Lysine ($^{13}C_6$, 99%) Irradiated Mouse Feed
MLK-LYS-C	MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed Kit Kit contains: 1 kg of L-Lysine $^{13}C_6$ feed and 1 kg of (unlabeled) feed
MLK-LYS-C-IR	MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed Kit, Irradiated Kit contains: 1 kg of L-Lysine $^{13}C_6$ feed and 1 kg of (unlabeled) feed

MouseExpress® Unlabeled Mouse Feed

Catalog No.	Description
MF-UNLABELED	MouseExpress® Unlabeled Mouse Feed
MF-UNLABELED-IR	MouseExpress® Unlabeled Irradiated Mouse Feed

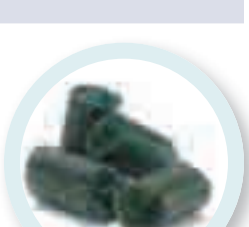


Custom-formulated amino acid-defined diets are available in additional labeling patterns and amino acid substitutions. Please inquire.

MouseExpress® is a registered trademark of Cambridge Isotope Laboratories, Inc.

MouseExpress® (^{15}N, 98%) Mouse Feed

Catalog No.	Description
MF-SPIRULINA-N	MouseExpress® (^{15}N, 98%) Mouse Feed prepared with Spirulina (U-^{15}N, 98%+)
MF-SPIRULINA-N-IR	MouseExpress® (^{15}N, 98%) Mouse Feed, Irradiated prepared with Spirulina (U-^{15}N, 98%+)
MF-SPIRULINA-U	MouseExpress® Unlabeled Mouse Feed prepared with unlabeled Spirulina
MF-SPIRULINA-U-IR	MouseExpress® Unlabeled Mouse Feed, Irradiated prepared with unlabeled Spirulina
MLK-SPIRULINA-N	MouseExpress® (^{15}N, 98%) Mouse Feed Kit prepared with Spirulina Kit contains: 1 kg Spirulina (^{15}N, 98%) feed and 1 kg Spirulina (unlabeled) feed
MLK-SPIRULINA-N-IR	MouseExpress® (^{15}N, 98%) Mouse Feed Kit, Irradiated prepared with Spirulina Kit contains: 1 kg Spirulina (^{15}N, 98%) feed and 1 kg Spirulina (unlabeled) feed



MouseExpress® L-Leucine (5,5,5-D_3, 98%) Mouse Feed

Catalog No.	Description
MF-LEU-D3	MouseExpress® L-Leucine (5,5,5-D_3, 98%) Mouse Feed
MF-LEU-D3-IR	MouseExpress® L-Leucine (5,5,5-D_3, 98%) Irradiated Mouse Feed
MLK-LEU-D3	MouseExpress® L-Leucine (5,5,5-D_3, 98%) Mouse Feed Kit Kit contains: 1 kg of L-Leucine D_3 feed and 1 kg of (unlabeled) feed
MLK-LEU-D3-IR	MouseExpress® L-Leucine (5,5,5-D_3, 98%) Mouse Feed Kit, Irradiated Kit contains: 1 kg of L-Leucine D_3 feed and 1 kg of (unlabeled) feed

We have used the MouseExpress® L-Lysine ($^{13}C_6$, 99%) Mouse Feed Labeling Kit from Cambridge Isotope Labs (CIL) to label a colony of C57BL6 mice. We achieved full labeling efficiency by F2 generation in the muscle tissue, our tissue of interest and in all other tissues tested. In addition, CIL's MouseExpress® (^{15}N, 98%) Mouse Feed was used in non-generational labeling of a colony of C57BL6 mice. We achieved full labeling efficiency after 12 weeks of feeding the MouseExpress® (^{15}N, 98%) Mouse Feed. These labeled tissues are fueling a variety of studies for multiple principle investigators at our research institute to study Duchenne muscular dystrophy, myositis, urea cycle disorders and vanishing white matter disease.

Kristy J. Brown, PhD, and Yetrib Hathout, PhD
Children's National Medical Center
Center for Genetic Medicine

MouseExpress® Mouse Tissue

MouseExpress® L-Lysine ($^{13}C_6$, 97%) Mouse Tissue

Catalog No.	Description
MT-LYSC6-MAW	MouseExpress® Abdominal Adipose Tissue (white) (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FAW	MouseExpress® Abdominal Adipose Tissue (white) (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MAB	MouseExpress® Interscapular Adipose Tissue (brown) (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FAB	MouseExpress® Interscapular Adipose Tissue (brown) (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MBL	MouseExpress® Bladder Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FBL	MouseExpress® Bladder Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MBR	MouseExpress® Breast Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FBR	MouseExpress® Breast Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MB	MouseExpress® Brain Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FB	MouseExpress® Brain Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MC	MouseExpress® Cecum Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FC	MouseExpress® Cecum Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MCO	MouseExpress® Colon Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FCO	MouseExpress® Colon Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MD	MouseExpress® Duodenum Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FD	MouseExpress® Duodenum Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MEY	MouseExpress® Eye Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FEY	MouseExpress® Eye Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MFB	MouseExpress® Femur Bone (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FFB	MouseExpress® Femur Bone (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MH	MouseExpress® Heart Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FH	MouseExpress® Heart Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MIL	MouseExpress® Ileum Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FIL	MouseExpress® Ileum Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-ME	MouseExpress® Inner Ear Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FE	MouseExpress® Inner Ear Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MI	MouseExpress® Intestine (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FI	MouseExpress® Intestine (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MJ	MouseExpress® Jejunum Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FJ	MouseExpress® Jejunum Tissue (F) L-Lysine ($^{13}C_6$, 97%)

MouseExpress® (^{15}N, 94%) Mouse Tissue

Catalog No.	Description
MT-15N-MB	MouseExpress® Brain Tissue (M) (^{15}N, 94%)
MT-15N-ML	MouseExpress® Liver Tissue (M) (^{15}N, 94%)
MT-15N-MM	MouseExpress® Muscle Tissue (M) (^{15}N, 94%)
MT-15N-MSE	MouseExpress® Serum (M) (^{15}N, 94%)
MT-15N-MSP	MouseExpress® Spleen Tissue (M) (^{15}N, 94%)
MT-15N-MTA	MouseExpress® Tail (M) (^{15}N, 94%)
MT-15N-MT	MouseExpress® Testis Tissue (M) (^{15}N, 94%)

Catalog No.	Description
MT-LYSC6-MK	MouseExpress® Kidney Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FK	MouseExpress® Kidney Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-ML	MouseExpress® Liver Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FL	MouseExpress® Liver Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MLU	MouseExpress® Lung Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FLU	MouseExpress® Lung Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MMAM	MouseExpress® Mammary Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FMAM	MouseExpress® Mammary Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MM	MouseExpress® Muscle Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FM	MouseExpress® Muscle Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FO	MouseExpress® Ovaries; Bilateral (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MP	MouseExpress® Pancreas Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FP	MouseExpress® Pancreas Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MPL	MouseExpress® Plasma (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FPL	MouseExpress® Plasma (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MSE	MouseExpress® Serum (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FSE	MouseExpress® Serum (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MSK	MouseExpress® Skin Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FSK	MouseExpress® Skin Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MSP	MouseExpress® Spleen Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FSP	MouseExpress® Spleen Tissue (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MSC	MouseExpress® Spinal Cord (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FSC	MouseExpress® Spinal Cord (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MST	MouseExpress® Stomach (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FST	MouseExpress® Stomach (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MT	MouseExpress® Testis Tissue (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MTB	MouseExpress® Tibia Bone (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FTB	MouseExpress® Tibia Bone (F) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-MTH	MouseExpress® Thymus (M) L-Lysine ($^{13}C_6$, 97%)
MT-LYSC6-FTH	MouseExpress® Thymus (F) L-Lysine ($^{13}C_6$, 97%)

M – Male F – Female

MS/MS Standards

Catalog No.	Description
NSK-A	Labeled Amino Acid Standards Set A
NSK-B	Labeled Carnitine Standards Set B
NSK-B-G	Labeled Carnitine Standards (supplement to NSK-B)
NSK-AB	Labeled Standards Sets A & B (2 x 10 vials)
NSK-T	Labeled Succinylacetone Standard – 1 Set T

Please see pages 110-111 for further information on the MS/MS standards.

Other Metabolites and Substrates

Catalog No.	Description
CLM-2436	Acetaminophen (carbonyl-^{13}C, 99%)
CLM-317	Acetic acid (1-^{13}C, 99%)
CLM-318	Acetic acid (2-^{13}C, 99%)
CLM-113	Acetic acid (1,2-$^{13}C_2$, 99%)
DLM-7476	ADMA•HCl•H_2O (Asymmetric dimethylarginine) (2,3,3,4,4,5,5-D_7, 98%)
CLM-630	Aminopyrine (*N,N*-dimethyl-$^{13}C_2$, 99%)
DLM-2762	Amitriptyline•HCl (*N*-methyl-D_3, 98%)
CLM-6585	Aspirin (acetyl-1-^{13}C, 99%)
CLM-3655	AZT (methyl-^{13}C, 99%) CP 96%
DLM-2790	Buspirone•HCl (butyl-D_8, 98%)
CLM-728	Caffeine (3-methyl-^{13}C, 99%)
NLM-332	Caffeine (1,3-$^{15}N_2$, 99%)
CNLM-333	Caffeine (2-^{13}C, 99%; 1,3-$^{15}N_2$, 98%+)
DLM-3555	L-Carnitine (trimethyl-D_9, 98%)
ULM-7801	L-Carnitine (unlabeled)
ULM-8247	L-Carnitine (mono), *O*-glutaryl, dibenzyl (unlabeled)
DLM-8275	L-Carnitine•ClO_4, benzyl ester (*N*-methyl-D_3, 98%)
DLM-1871	L-Carnitine•HCl (methyl-D_3, 98%)
DLM-3820	L-Carnitine•HCl (dimethyl-D_6, 98%)
DLM-754	L-Carnitine•HCl, *O*-acetyl (*N*-methyl-D_3, 98%)
DLM-3821	L-Carnitine•HCl, *O*-acetyl (*N,N*-dimethyl-D_6, 98%) CP 97%
DLM-3822	L-Carnitine•HCl, *O*-acetyl (*N,N,N*-trimethyl-D_9, 98%) CP 95%+
ULM-7802	L-Carnitine•HCl, *O*-acetyl (unlabeled)
DLM-3861	L-Carnitine•HCl, *O*-butyryl (*N*-methyl-D_3, 98%)
ULM-7704	L-Carnitine•HCl, *O*-butyryl (unlabeled)
ULM-7195	L-Carnitine•HCl, *O*-decanoyl (unlabeled)
DLM-8162	L-Carnitine•HCl, *O*-dodecanoyl (*N*-methyl-D_3, 98%)
DLM-6718	L-Carnitine•HCl, *O*-hexacosanoyl (*N*-methyl-D_3, 98%) CP 80%
ULM-6719	L-Carnitine•HCl, *O*-hexacosanoyl (unlabeled) CP 80%
ULM-7198	L-Carnitine•HCl, *O*-hexanoyl (unlabeled)
DLM-3974	L-Carnitine•HCl, *O*-isovaleryl (*N,N,N*-trimethyl-D_9, 98%)
ULM-4697	L-Carnitine•HCl, *O*-isovaleryl (unlabeled)
DLM-4425	L-Carnitine•HCl, *O*-myristoyl (*N,N,N*-trimethyl-D_9, 98%)
ULM-7737	L-Carnitine•HCl, *O*-myristoyl (unlabeled)
DLM-755	L-Carnitine•HCl, *O*-octanoyl (*N*-methyl-D_3, 98%)
ULM-7770	L-Carnitine•HCl, *O*-octanoyl (unlabeled)
DLM-1263	L-Carnitine•HCl, *O*-palmitoyl (*N*-methyl-D_3, 98%)
ULM-7738	L-Carnitine•HCl, *O*-palmitoyl (unlabeled)
DLM-3973	L-Carnitine•HCl, *O*-propionyl (*N*-methyl-D_3, 98%)
ULM-7705	L-Carnitine•HCl, *O*-propionyl (unlabeled)
CLM-6126	β-Carotene (10,10′,11,11′-$^{13}C_4$, 99%)
DLM-3829	β-Carotene (19,19,19,19′,19′,19′-D_6, 98%)
DLM-2439	β-Carotene (10,10′,19,19,19,19′,19′,19′-D_8, 97%)
CLM-548	Choline chloride (1,2-$^{13}C_2$, 99%)
CLM-1608	Chloral hydrate (trichloromethyl-^{13}C, 97%)
CLM-4415	Citric acid (3-^{13}C, 99%)
CLM-7337	Citric acid (1,5-$^{13}C_2$, 99%)
CLM-148	Citric acid (2,4-$^{13}C_2$, 99%)
CLM-9021	Citric acid ($^{13}C_6$, 99%) CP 97%
DLM-3487	Citric acid (2,2,4,4-D_4, 98%)
DLM-1287	Clonidine•HCl (4,4,5,5-imidazoline-D_4, 98%)
DLM-2816	Clozapine (4-methylpiperazinyl-D_4, 97%)

Catalog No.	Description
DLM-1819	DL-Cotinine (methyl-D_3, 98%)
CLM-7933	Creatine (guanidino-^{13}C, 99%)
DLM-7504	Dexamethasone (4,6-α,21,21-D_4, 96%) (18% D at C_2 position)
DLM-1886	Diazepam (phenyl-D_5, 98%)
CLM-7401	L-Dopa (1-^{13}C, 99%)
CLM-3723	L-Dopa (alkyl-2,3-$^{13}C_2$, 98%)
CLM-2231	L-Dopa (ring-3,5-$^{13}C_2$, 99%)
CLM-1007	L-Dopa (ring-$^{13}C_6$, 99%)
CLM-3724	L-Dopa (ring-$^{13}C_6$, alkyl-2,3-$^{13}C_2$, 99%)
DLM-2084	L-Dopa (ring-D_3, 98%)
COLM-2232	L-Dopa (2,3-$^{13}C_2$, 97%; 4-hydroxy-^{18}O, 95%)
COLM-2233	L-Dopa (ring-$^{13}C_6$, 99%; 4-hydroxy-^{18}O, 95%)
CLM-3368	Dopamine•HCl (1-^{13}C, 99%)
CLM-3369	Dopamine•HCl (ring-$^{13}C_6$, 99%)
DLM-2833	Dopamine•HCl (1,1-D_2, 93%) CP 96-98%
DLM-2834	Dopamine•HCl (2,2-D_2, 97-98%)
DLM-2181	Dopamine•HCl (ring-D_3, 98%)
DLM-2498	Dopamine•HCl (1,1,2,2-D_4, 97-98%)
CNLM-3445	Dopamine•HCl (1-^{13}C, 99%; ^{15}N, 99%)
DLM-2290	Dopamine•HCl (ring-D_3, 97%; 2,2-D_2, 97%)
DLM-2744	Enalaprilat•H_2O (phenyl-D_5, 98%)
DLM-2745	Enalapril maleate (phenyl-D_5, 98%)
CLM-123	Erythromycin (*N*-methyl-^{13}C, 99%)
CLM-165	Erythromycin lactobionate salt (*N*-methyl-^{13}C, 99%)
CLM-3758	Erythromycin lactobionate salt (*N,N*-dimethyl-$^{13}C_2$, ~90%)
CLM-344	Ethanol (1-^{13}C, 99%) (<6% H_2O)
CLM-130	Ethanol (2-^{13}C, 99%) (<6% H_2O)
CLM-551	Ethanol (1,2-$^{13}C_2$, 99%) (<6% H_2O)
CLM-2291	Ethanolamine ($^{13}C_2$, 99%)
CLM-3911	Ethanolamine•HCl (1-^{13}C, 99%)
CLM-274	Ethanolamine•HCl (1,2-$^{13}C_2$, 99%)
CLM-522	Ethyl acetoacetate (1,3-$^{13}C_2$, 99%)
CLM-523	Ethyl acetoacetate (2,4-$^{13}C_2$, 99%)
CLM-1529	Fumaric acid ($^{13}C_4$, 99%)
DLM-1539	Fumaric acid (2,3-D_2, 98%)
DLM-7654	Fumaric acid (D_4, 98%)
CDLM-8473	Fumaric acid (1,4-$^{13}C_2$, 99%; 2,3-D_2, 98%)
CDLM-6062	Fumaric acid (1-^{13}C, 99%; 2,3-D_2, 98%)
DLM-3996	Glybenclamide (cyclohexylamine-D_{11}, 98%)
CLM-1397	Glycerol (2-^{13}C, 99%)
CLM-1857	Glycerol (1,3-$^{13}C_2$, 99%)
CLM-1510	Glycerol ($^{13}C_3$, 99%)
DLM-1229	Glycerol (1,1,2,3,3-D_5, 99%)
DLM-558	Glycerol (D_8, 99%)
CDLM-7745	Glycerol ($^{13}C_3$, 99%; D_8, 98%) CP 95%
DLM-2911	Histamine•2HCl (α,α,β,β-D_4, 98%)
CLM-8042	Hypoxanthine ($^{13}C_5$, 99%)
DLM-8658	Hypoxanthine (2,8-D_2, 98%)
DLM-2923	Hypoxanthine (2,3,7,8-D_4, 98%)
NLM-8500	Hypoxanthine ($^{15}N_4$, 98%)
CNLM-7894	Hypoxanthine ($^{13}C_5$, 99%; $^{15}N_4$, 98%)
DLM-3035	Imipramine•HCl (2,4,6,8-D_4, 98%) CP 97%
CLM-7118	Ketoconazole (carbonyl-^{13}C, 99%)

CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

Other Metabolites and Substrates

Catalog No.	Description
CLM-2411	α-Ketoglutaric acid (U-$^{13}C_5$, 99%)
CLM-4442	α-Ketoglutaric acid, disodium salt (1,2,3,4-$^{13}C_4$, 99%) CP 97%
CLM-8468	α-Ketoisocaproic acid (1-^{13}C, 99%)
CLM-2093	α-Ketoisocaproic acid, sodium salt (1-^{13}C, 99%)
CLM-7742	α-Ketoisocaproic acid, sodium salt (2-^{13}C, 99%)
DLM-1944	α-Ketoisocaproic acid, sodium salt (methyl-D_3, 98%)
DLM-4214	α-Ketoisocaproic acid, sodium salt (isopropyl-D_7, 98%)
CLM-7613	*trans*-Lycopene (8,8′,9,9′,10,10′,11,11′,19,19′-$^{13}C_{10}$, 99%)
DLM-1129	Maleic acid (2,3-D_2 , 98%)
CLM-310	Maleic anhydride (1,4-$^{13}C_2$, 99%)
CLM-312	Maleic anhydride (2,3-$^{13}C_2$, 99%)
CLM-6019	Maleic anhydride ($^{13}C_4$, 99%)
DLM-7101	Melatonin (acetyl-D_3, 98%)
CLM-1280	Methacetin (methoxy-^{13}C, 99%)
DLM-2646	5-Methoxytryptamine•HCl (α,α,β,β-D_4, 98%)
DLM-651	Methyl formate (formyl-D, 99%)
CLM-7522	Naproxen, sodium salt (*O*-methyl-^{13}C, 98%)
DLM-6883	Nicotinamide (D_4, 98%)
CLM-3914	DL-Nicotine (3′,4′,5′-$^{13}C_3$, 99%)
DLM-1818	DL-Nicotine (methyl-D_3, 98%)
CLM-4892	DL-Nornicotine (3′,4′,5′-$^{13}C_3$, 99%)
DLM-9017	DL-Nornicotine (pyridine-D_4, 98%)
NLM-1048	Orotic acid•H_2O (1,3-$^{15}N_2$, 98%+)
DLM-1888	Oxazepam (phenyl-D_5, 98%)
CLM-1296	Phenacetin (ethoxy-1-^{13}C, 99%)
CLM-432	Phenobarbital (2,4,5-$^{13}C_3$, 90%)
DLM-433	Phenobarbital (ethyl-D_5, 98%)
DLM-1688	Phenobarbital (ring-D_5, 99%)
CNLM-110	Phenobarbital (2-^{13}C, 99%; 1,3-$^{15}N_2$, 99%)
CLM-3551	Potassium phosphoenol pyruvate (2-^{13}C, 99%)
CLM-2723	Potassium phosphoenol pyruvate (3-^{13}C, 99%)
CLM-3398	Potassium phosphoenol pyruvate (2,3-$^{13}C_2$, 99%)
DLM-3041	Primidone (ethyl-D_5, 98%)
CLM-646	Propionic acid (1-^{13}C, 99%)
CLM-647	Propionic acid ($^{13}C_3$, 99%)
CLM-8077	Pyruvic acid (1-^{13}C, 99%)
CLM-8849	Pyruvic acid (2-^{13}C, 99%) CP 95%
DLM-2659	DL-Secobarbital (1-methyl-D_3, butyl-2,2-D_2, 98%)
DLM-3579	Serotonin creatinine sulfate complex (α,α,β,β-D_4, 98%)
CLM-156	Sodium acetate (1-^{13}C, 99%)
CLM-381	Sodium acetate (2-^{13}C, 99%)
CLM-440	Sodium acetate (1,2-$^{13}C_2$, 99%)
DLM-3165	Sodium acetate (D_3, 90%)
DLM-3126	Sodium acetate (D_3, 99%)
OLM-1077	Sodium acetate ($^{18}O_2$, 95%)
CDLM-611	Sodium acetate (1-^{13}C, 99%; D_3, 98%)
CDLM-1240	Sodium acetate (2-^{13}C, 99%; D_3, 98%)
CDLM-3457	Sodium acetate (1,2-$^{13}C_2$, 99%; D_3, 98%)
COLM-1230	Sodium acetate (1-^{13}C, 99%; $^{18}O_2$, 96%)
CLM-441	Sodium bicarbonate (^{13}C, 99%) CP 97%+
CLM-1256	Sodium butyrate (1-^{13}C, 99%)

Catalog No.	Description
CLM-3706	Sodium D-3-hydroxybutyrate (2,4-$^{13}C_2$, 99%)
DLM-7644	Sodium D-3-hydroxybutyrate (4,4,4-D_3, 99%)
CLM-3634	Sodium DL-3-hydroxybutyrate (2,4-$^{13}C_2$, 99%)
CLM-3780	Sodium dichloroacetate ($^{13}C_2$, 99%)
CLM-583	Sodium formate (^{13}C, 99%)
CLM-1577	Sodium L-lactate (1-^{13}C, 99%) (20% w/w in H_2O)
CLM-1578	Sodium L-lactate (3-^{13}C, 98%) (20% w/w in H_2O)
CLM-1579	Sodium L-lactate ($^{13}C_3$, 98%) (20% w/w in H_2O)
DLM-3317	Sodium L-lactate (3,3,3-D_3, 98%) (20% w/w in H_2O)
CLM-771	Sodium propionate (1-^{13}C, 99%)
CLM-1506	Sodium propionate (2-^{13}C, 99%)
CLM-4573	Sodium propionate (3-^{13}C, 99%)
CLM-3042	Sodium propionate (2,3-$^{13}C_2$, 99%)
CLM-1865	Sodium propionate ($^{13}C_3$, 99%)
CLM-1082	Sodium pyruvate (1-^{13}C, 99%)
CLM-1580	Sodium pyruvate (2-^{13}C, 99%)
CLM-1575	Sodium pyruvate (3-^{13}C, 99%)
CLM-3507	Sodium pyruvate (2,3-$^{13}C_2$, 99%)
CLM-2440	Sodium pyruvate ($^{13}C_3$, 99%)
DLM-6068	Sodium pyruvate (D_3, 97-98%)
CDLM-6063	Sodium pyruvate (2-^{13}C, 99%; D_3, 98%)
DLM-7311	Stearoyl coenzyme A (stearoyl-methyl-D_3, 98%) CP 90%
CLM-1084	Succinic acid (1,4-$^{13}C_2$, 99%)
CLM-1199	Succinic acid (2,3-$^{13}C_2$, 99%)
CLM-1571	Succinic acid ($^{13}C_4$, 99%)
DLM-584	Succinic acid (D_4, 98%)
DLM-831	Succinic acid (D_6, 98%)
CDLM-7754	Succinic acid ($^{13}C_4$, 99%; 2,2,3,3-D_4, 98%)
DLM-2307	Succinic acid, disodium salt (D_4, 75%)
CLM-6622	Taurine (1,2-$^{13}C_2$, 98%)
NLM-4472	Taurine (^{15}N, 98%+)
CLM-7119	Temozolomide (methyl-^{13}C, 99%)
CLM-7491	*cis*-(+/-)-Tramadol•HCl (methoxy-^{13}C, 99%)
DLM-6989	Tryptamine (α,α,β,β-D_4, 97%)
DLM-2919	Tyramine (1,1,2,2-D_4, 98%)
DLM-8075	Tyramine•HCl (1,1,2,2-D_4, 98%)
NLM-637	Uracil (1,3-$^{15}N_2$, 98%)
CLM-311	Urea (^{13}C, 99%)
DLM-1269	Urea (D_4, 98%)
NLM-232	Urea ($^{15}N_1$, 98%+)
NLM-233	Urea ($^{15}N_2$, 98%+)
OLM-655	Urea (^{18}O, 95%)
CNLM-234	Urea (^{13}C, 99%; $^{15}N_2$, 98%+)
COLM-4861	Urea (^{13}C, 99%; ^{18}O, 98%)
NOLM-654	Urea ($^{15}N_2$, 99%; ^{18}O, 96-99%)
CNOLM-8871	Urea (^{13}C, 99%; $^{15}N_2$, 99%; ^{18}O, 99%)
CLM-3399	Valproic acid (1,2,3,3′-$^{13}C_4$, 99%)
DLM-4291	Valproic acid (4,4,4′,4′-D_4, 98%)
DLM-7876	Valproic acid (propyl-1,1-D_2, pentanoic-3,3-D_2, 98%)
DLM-8875	Valproic acid (D_{15}, 98%)
NLM-1698	Xanthine (1,3-$^{15}N_2$, 98%+) CP 90%

CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

Pharmaceutical and Personal Care Products (PPCPs)

Catalog No.	Description
CNLM-3726-1.2	Acetaminophen (acetyl-$^{13}C_2$, 99%; ^{15}N, 98%+) 100 µg/mL in acetonitrile
ULM-7629-1.2	Acetaminophen (unlabeled) 100 µg/mL in acetonitrile
DLM-3008-1.2	Amitriptyline•HCl (*N,N*-dimethyl-D_6, 98%) 100 µg/mL in methanol
ULM-8350-1.2	Amitriptyline•HCl (unlabeled) 100 µg/mL in methanol
CLM-7407-1MG	Amoxicillin•3H_2O (phenyl-$^{13}C_6$, 99%)
DLM-183-1.2	Benzophenone (D_{10}, 98%) 100 µg/mL in nonane
ULM-8303-1.2	Benzophenone (unlabeled) 100 µg/mL in nonane
CLM-4325-1.2	Bisphenol A (ring-$^{13}C_{12}$, 99%) 100 µg/mL in acetonitrile
ULM-7106-1.2	Bisphenol A (unlabeled) 100 µg/mL in acetonitrile
CLM-8285-1.2	*n*-Butyl paraben (ring-$^{13}C_6$, 99%) 1mg/mL in methanol
ULM-8287-1.2	*n*-Butyl paraben (unlabeled) 1 mg/mL in methanol
CLM-514-1.2	Caffeine (trimethyl-$^{13}C_3$, 99%) 100 µg/mL in methanol
ULM-7653-1.2	Caffeine (unlabeled) 100 µg/mL in methanol
DLM-2806-1.2	Carbamazepine (D_{10}, 98%) 100 µg/mL in acetonitrile-D_3
ULM-6581-1.2	Carbamazepine (unlabeled) 100 µg/mL in acetonitrile
DLM-119-1.2	(+/-)-Chloramphenicol (ring-D_4, benzyl-D_1, 98%) 100 µg/mL in acetonitrile
ULM-6687-1.2	(+/-)-Chloramphenicol (unlabeled) 100 µg/mL in acetonitrile
CNLM-7539-1.2	Ciprofloxacin•HCl (2,3,carboxyl-$^{13}C_3$, 99%; quinoline-^{15}N, 98%) 100 µg/mL in methanol
ULM-7710-1.2	Ciprofloxacin•HCl monohydrate (unlabeled) 100 µg/mL in methanol
DLM-2218-0.1MG	Cortisol (9,11,12,12-D_4, 98%)
ULM-7823-0.1MG	Cortisol (unlabeled)
CLM-8569-1.2	Dechlorane Plus *syn* (*bis*-cyclopentene-$^{13}C_{10}$, 99%) 100 µg/mL in nonane
CLM-8569-T-1.2	Dechlorane Plus *syn* (*bis*-cyclopentene-$^{13}C_{10}$, 99%) 100 µg/mL in toluene
ULM-7886-1.2	Dechlorane Plus *syn* (unlabeled) 100 µg/mL in nonane
ULM-7886-T-1.2	Dechlorane Plus *syn* (unlabeled) 100 µg/mL in toluene
CLM-8588-1.2	Dechlorane Plus *anti* (*bis*-cyclopentene-$^{13}C_{10}$, 99%) 100 µg/mL in nonane
CLM-8588-T-1.2	Dechlorane Plus *anti* (*bis*-cyclopentene-$^{13}C_{10}$, 99%) 100 µg/mL in toluene
ULM-7887-1.2	Dechlorane Plus *anti* (unlabeled) 100 µg/mL in nonane
ULM-7887-T-1.2	Dechlorane Plus *anti* (unlabeled) 100 µg/mL in toluene
ULM-7777-1.2	Dechlorane Plus Technical Product (unlabeled) 100 µg/mL in nonane
DLM-8567-1.2	Diclofenac (phenyl-D_4, 98%) 100 µg/mL in methylene chloride CP 96%
ULM-9023-1.2	Diclofenac (unlabeled) 100 µg/mL in methylene chloride
DLM-1632-1.2	Diethylene glycol (D_8, 98%) 1 mg/mL in methanol
ULM-8235-1.2	Diethylene glycol (unlabeled) 1 mg/mL in methanol
DLM-170-1.2	Diethylstilbestrol (*cis*/*trans* mix) 100 µg/mL in CD_2Cl_2 (ring-3,3′,5,5′-diethyl-1,1,1′,1′-D_8, 98%)
DLM-170-D-1.2	Diethylstilbestrol (*cis*/*trans* mix) 100 µg/mL in *p*-dioxane (ring-3,3′,5,5′-diethyl-1,1,1′,1′-D_8, 98%)
ULM-7921-1.2	Diethylstilbestrol (*cis*/*trans* mix) (unlabeled) 100 µg/mL in methylene chloride
ULM-7921-D-1.2	Diethylstilbestrol (*cis*/*trans* mix) (unlabeled) 100 µg/mL in *p*-dioxane

Catalog No.	Description
CNLM-411-1.2	5,5-Diphenylhydantoin (2-^{13}C, 99%; 1,3-$^{15}N_2$, 98%) 100 µg/mL in methanol
ULM-8533-1.2	5,5-Diphenylhydantoin (unlabeled) 100 µg/mL in methanol
CLM-3672-1.2	Erythromycin (90-95% Erythromycin A) (*N,N*-dimethyl-$^{13}C_2$, ~90%) 100 µg/mL in acetonitrile
ULM-4322-1.2	Erythromycin (unlabeled) 100 µg/mL in acetonitrile
CLM-803-1.2	Estradiol (3,4-$^{13}C_2$, 99%) 100 µg/mL in acetonitrile
CLM-7936-1.2	DL-Estradiol (13,14,15,16,17,18-$^{13}C_6$, 99%) 100 µg/mL in methanol
ULM-7449-1.2	Estradiol (unlabeled) 100 µg/mL in acetonitrile
DLM-7468-1.2	Estriol (2,4-D_2, 98%) 100 µg/mL in *p*-dioxane
ULM-8218-1.2	Estriol (unlabeled) 100 µg/mL in *p*-dioxane
CLM-7935-1.2	DL-Estrone (13,14,15,16,17,18-$^{13}C_6$, 99%) 100 µg/mL in methanol
ULM-7212-1.2	Estrone (unlabeled) 100 µg/mL in acetonitrile
CLM-3375-1.2	Ethynylestradiol (20,21-$^{13}C_2$, 99%) 100 µg/mL in acetonitrile
ULM-7211-1.2	Ethynylestradiol (unlabeled) 100 µg/mL in acetonitrile
DLM-8221-1.2	Gemfibrozil (2,2-dimethyl-D_6, 98%) 100 µg/mL in *p*-dioxane
ULM-8225-1.2	Gemfibrozil (unlabeled) 100 µg/mL in *p*-dioxane
CLM-4748-1.2	1,6-Anhydro-β-D-glucose (Levoglucosan) (U-$^{13}C_6$, 98%) 100 µg/mL in dimethyl sulfoxide
ULM-8000-1.2	1,6-Anhydro-β-D-glucose (Levoglucosan) (unlabeled) 100 µg/mL in dimethyl sulfoxide
CLM-8008-1.2	Hexachlorophene ($^{13}C_{13}$, 99%) 50 µg/mL in methanol
ULM-8009-1.2	Hexachlorophene (unlabeled) 50 µg/mL in methanol
CLM-4745-1.2	4-Hydroxybenzoic acid (ring-$^{13}C_6$, 99%) 1 mg/mL in methanol
ULM-8251-1.2	4-Hydroxybenzoic acid (unlabeled) 1 mg/mL in methanol
CLM-6779-1.2	2′,4,4′-Trichloro-2-hydroxydiphenyl ether ($^{13}C_{12}$, 99%) (Triclosan) 100 µg/mL in nonane
ULM-6935-1.2	2′,4,4′-Trichloro-2-hydroxydiphenyl ether (unlabeled) (Triclosan) 100 µg/mL in nonane
CLM-8012-0.1MG	DL-2-Hydroxyestradiol (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8135-0.1MG	2-Hydroxyestradiol (unlabeled)
CLM-8013-0.1MG	DL-4-Hydroxyestrone (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8261-0.1MG	4-Hydroxyestrone (unlabeled) CP 96%
ULM-8134-0.1MG	2-Hydroxyestrone (unlabeled)
CLM-8016-0.1MG	DL-2-Hydroxyestrone-3-methyl ether (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8133-0.1MG	2-Hydroxyestrone-3-methyl ether (unlabeled)
CLM-6943-1.2	Ibuprofen (propionic-$^{13}C_3$, 99%) 100 µg/mL in acetonitrile
ULM-7275-1.2	Ibuprofen (unlabeled) 100 µg/mL in acetonitrile
CLM-8015-0.1MG	DL-2-Methoxyestradiol (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8137-0.1MG	2-Methoxyestradiol (unlabeled)
CLM-8014-0.1MG	DL-2-Methoxyestrone (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8263-0.1MG	2-Methoxyestrone (unlabeled)
CLM-8017-0.1MG	DL-4-Methoxyestrone (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8262-0.1MG	4-Methoxyestrone (unlabeled)
DLM-4766-1.2	2-Methylisoborneol (2-methyl-D_3, 98%) (unlabeled) 100 µg/mL in nonane

Pharmaceutical and Personal Care Products (PPCPs)

Catalog No.	Description
CLM-8249-1.2	Methyl paraben (methyl 4-hydroxybenzoate) (ring-$^{13}C_6$, 99%) 1 mg/mL in methanol
ULM-8250-1.2	Methyl paraben (methyl 4-hydroxybenzoate) (unlabeled) 1 mg/mL in methanol
CLM-7885-1.2	Methyl Triclosan (2,4,4′-Trichloro-2′-methoxydiphenyl ether) (ring-$^{13}C_{12}$, 99%)100 µg/mL in nonane
ULM-7884-1.2	Methyl Triclosan (2,4,4′-Trichloro-2′-methoxydiphenyl ether) (unlabeled) 100 µg/mL in nonane
CDLM-7665-1.2	Naproxen 100 µg/mL in acetonitrile (*O*-methyl-^{13}C, 99%; *O*-methyl-D_3, 98%)
ULM-7709-1.2	Naproxen (unlabeled) 100 µg/mL in acetonitrile
CLM-4306-1.2	*p-n*-Nonylphenol (ring-$^{13}C_6$, 99%) 100 µg/mL in nonane
ULM-4559-1.2	*p-n*-Nonylphenol (unlabeled) 100 µg/mL in nonane
ULM-6560-1.2	*p*-Nonylphenol – technical grade (unlabeled) 100 µg/mL in nonane
CLM-4307-1.2	*p-n*-Nonylphenol diethoxylate (ring-$^{13}C_6$, 99%) 100 µg/mL in nonane
ULM-4521-1.2	*p-n*-Nonylphenol diethoxylate (unlabeled) 100 µg/mL in nonane
ULM-4521-SA-5X-1.2	*p-n*-Nonylphenol diethoxylate (unlabeled) 500 µg/mL in acetonitrile
ULM-7147-1.2	Nonylphenol diethoxylate – branched isomers (unlabeled) 100 µg/mL in nonane
CLM-4512-1.2	*p-n*-Nonylphenol monoethoxylate (ring-$^{13}C_6$, 99%) 100 µg/mL in nonane
ULM-4520-1.2	*p-n*-Nonylphenol monoethoxylate (unlabeled) 100 µg/mL in nonane
ULM-4520-SA-5X-1.2	*p-n*-Nonylphenol monoethoxylate (unlabeled) 500 µg/mL in acetonitrile
ULM-7146-1.2	Nonylphenol monoethoxylate – branched isomers (unlabeled) 100 µg/mL in nonane
CLM-4516-1.2	*p-n*-Nonylphenol triethoxylate (ring-$^{13}C_6$, 99%) 100 µg/mL in nonane
CLM-8525-1.2	Oxybenzone (phenyl-$^{13}C_6$, 99%) 100 µg/mL in acetonitrile
ULM-8531-1.2	Oxybenzone (unlabeled) 100 µg/mL in acetonitrile
OLM-7310-1.2	Perchloric acid, sodium salt ($^{18}O_4$, 90%+) 100 µg/mL in water
ULM-7312-1.2	Perchloric acid, sodium salt (unlabeled) 100 µg/mL in water

Branched Nonylphenols

CLM-8356	4-(1,3-Dimethyl-1-ethylpentyl) phenol (ring-$^{13}C_6$, 99%)
ULM-8360	4-(1,3-Dimethyl-1-ethylpentyl) phenol (unlabeled)
CLM-8357-1.2	4-(1,4-Dimethyl-1-ethylpentyl) phenol (ring-$^{13}C_6$, 99%)
ULM-8361-1.2	4-(1,4-Dimethyl-1-ethylpentyl) phenol (unlabeled)
CLM-8358	4-(1,1,5-Trimethylhexyl) phenol (ring-$^{13}C_6$, 99%)
ULM-8362	4-(1,1,5-Trimethylhexyl) phenol (unlabeled)
CLM-8359-1.2	4-(1-Ethyl-1-methylhexyl) phenol (ring-$^{13}C_6$, 99%)
ULM-8363-1.2	4-(1-Ethyl-1-methylhexyl) phenol (unlabeled)

Catalog No.	Description
DLM-3039-1MG	Phenylbutazone (diphenyl-D_{10}, 98%)
ULM-7378-1MG	Phenylbutazone (unlabeled)
CLM-3733-1.2	*o*-Phenylphenol (phenyl-$^{13}C_6$, 99%) 100 µg/mL in nonane
ULM-7396-1.2	*o*-Phenylphenol (unlabeled) 100 µg/mL in nonane
OLM-8283-180-1.2	Potassium bromate ($^{18}O_3$, 98%) 100 µg/mL in $H_2{}^{18}O$
ULM-8451-1.2	Potassium bromate (unlabeled) 100 µg/mL in water
DLM-7953-1.2	Progesterone (2,2,4,6,6 17′,21,21,21-D_9, 98% 100 µg/mL in *p*-dioxane
ULM-8219-1.2	Progesterone (unlabeled) 100 µg/mL in *p*-dioxane
ULM-8654-1.2	2-(2-Hydroxyphenyl)-2-(4-hydroxyphenyl) propane (unlabeled) (2,4′-bisphenol A) 100 µg/mL in acetonitrile
DLM-4633-1.2	3-Chloro-1,2-propanediol (propane-D_5, 98%) CP 95% 1 mg/mL in methanol
ULM-7998-1.2	3-Chloro-1,2-propanediol (unlabeled) 1 mg/mL in methanol
CLM-7892	Resorcinol ($^{13}C_6$, 99%)
CLM-3045-1.2	Sulfamethazine (phenyl-$^{13}C_6$, 90%) 100 µg/mL in acetonitrile
ULM-7220-1.2	Sulfamethazine (unlabeled) 100 µg/mL in acetonitrile
CLM-6944-1.2	Sulfamethoxazole (ring-$^{13}C_6$, 99%) 100 µg/mL in acetonitrile
ULM-7527-1.2	Sulfamethoxazole (unlabeled) 100 µg/mL in acetonitrile
DLM-683-1.2	Testosterone (1,2-D_2, 98%) 100 µg/mL in methylene chloride
DLM-8085-1.2	Testosterone (2,2,4,6,6-D_5, 98%) 100 µg/mL in methylene chloride
DLM-8085-D-1.2	Testosterone (D_5, 98%) 100 µg/mL in *p*-dioxane
ULM-8081-1.2	Testosterone (unlabeled) 100 µg/mL in methylene chloride
ULM-8081-D-1.2	Testosterone (unlabeled) 100 µg/mL in *p*-dioxane
CLM-8370-1.2	Thiabendazole (ring-$^{13}C_6$, 99%) 100 µg/mL in acetonitrile
ULM-8371-1.2	Thiabendazole (unlabeled) 100 µg/mL in acetonitrile
DLM-6083-1.2	2,4,6-Trichloroanisole (D_5, 98%) 1 mg/mL in methanol-D
ULM-7999-1.2	2,4,6-Trichloroanisole (unlabeled) 1 mg/mL in methanol
CLM-7286-1.2	3,4,4′-Trichlorocarbanilide (Triclocarban) (4′-chlorophenyl-$^{13}C_6$, 99%) 100 µg/mL in acetonitrile
ULM-7968-1.2	3,4,4′-Trichlorocarbanilide (Triclocarban) (unlabeled) 100 µg/mL in acetonitrile
CLM-7988-A-1.2	Trimethoprim ($^{13}C_3$, 99%) 50 µg/mL in methanol
ULM-7989-A-1.2	Trimethoprim (unlabeled) 50 µg/mL in methanol
DLM-6861-1.2	Warfarin (phenyl-D_5, 98%) 100 µg/mL in acetonitrile-D_3
ULM-7242-1.2	Warfarin (unlabeled) 100 µg/mL in acetonitrile

As new analytes are frequently added to this list, please consult the CIL website, www.isotope.com, to see if desired target analytes have been added since this catalog, or contact envsales@isotope.com for a quotation.

Phthalate and Phthalate Metabolite Standards

Catalog No.	Description
DLM-1369-1.2	Benzyl butyl phthalate (ring-D_4, 98%) 100 µg/mL in nonane
ULM-7551-1.2	Benzyl butyl phthalate (unlabeled) 100 µg/mL in nonane
CLM-4675-1.2	Bis(2-ethylhexyl) adipate (adipate-$^{13}C_6$, 99%) 100 µg/mL in nonane
ULM-6566-1.2	Bis(2-ethylhexyl) adipate (unlabeled) 100 µg/mL in nonane
DLM-1368-1.2	Bis(2-ethylhexyl) phthalate (ring-D_4, 98%) 100 µg/mL in nonane
ULM-6241-1.2	Bis(2-ethylhexyl) phthalate (unlabeled) 1000 µg/mL in nonane
DLM-1367-1.2	Di-*n*-butyl phthalate (ring-D_4, 98%) 100 µg/mL in nonane
ULM-7466-1.5	Di-*n*-butyl phthalate (unlabeled) 100 µg/mL in nonane
CLM-4670-1.2	Dicyclohexyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in nonane
ULM-8785-1.2	Dicyclohexyl phthalate (unlabeled) 100 µg/mL in nonane
DLM-1629-1.2	Diethyl phthalate (ring-D_4, 98%) 100 µg/mL in nonane
ULM-6174-1.2	Diethyl phthalate (unlabeled) 100 µg/mL in nonane
CLM-4669-1.2	Di-*n*-hexyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in nonane
ULM-7434-1.2	Di-*n*-hexyl phthalate (unlabeled) 100 µg/mL in nonane
DLM-1366-1.2	Dimethyl phthalate (ring-D_4, 98%) 100 µg/mL in nonane
ULM-6783-1.2	Dimethyl phthalate (unlabeled) 100 µg/mL in nonane
DLM-1630-1.2	Di-*n*-octyl phthalate (ring-D_4, 98%) 100 µg/mL in nonane
ULM-6129-1.2	Di-*n*-octyl phthalate (unlabeled) 100 µg/mL in nonane
CLM-4668-1.2	Di-*n*-pentyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in nonane
ULM-7433-1.2	Di-*n*-pentyl phthalate (unlabeled) 100 µg/mL in nonane
CLM-4591-MT-1.2	Monobenzyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-6149-MT-1.2	Monobenzyl phthalate (unlabeled) 100 µg/mL in MTBE
CLM-6148-MT-1.2	Mono-*n*-butyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-6148-MT-1.2	Mono-*n*-butyl phthalate (unlabeled) 100 µg/mL in MTBE
CLM-8148-MT-1.2	Mono-(2-ethyl-5-carboxy-pentyl) phthalate (DEHP Metabolite V) ($^{13}C_4$, 99%) 100 µg/mL in MTBE
ULM-8149-MT-1.2	Mono-(2-ethyl-5-carboxy-pentyl) phthalate (DEHP Metabolite V) (unlabeled) 100 µg/mL in MTBE
CLM-8232-MT-1.2	Mono-[(2-carboxymethyl) hexyl] phthalate (DEHP Metabolite IV) ($^{13}C_4$, 99%) 100 µg/mL in MTBE
ULM-8233-MT-1.2	Mono-[(2-carboxymethyl) hexyl] phthalate (DEHP Metabolite IV) (unlabeled) 100 µg/mL in MTBE

Catalog No.	Description
CLM-6847-MT-1.2	Mono-(3-carboxypropyl) phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-6848-MT-1.2	Mono-(3-carboxypropyl) phthalate (unlabeled) 100 µg/mL in MTBE
CLM-4592-MT-1.2	Monocyclohexyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-7394-MT-1.2	Monocyclohexyl phthalate (unlabeled) 100 µg/mL in MTBE
CLM-4584-MT-1.2	Mono-2-ethylhexyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-4583-MT-1.2	Mono-2-ethylhexyl phthalate (unlabeled) 100 µg/mL in MTBE
CLM-6641-MT-1.2	Mono-(2-ethyl-5-hydroxyhexyl) phthalate (DEHP Metabolite IX) (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C^2$, 99%) 100 µg/mL in MTBE
ULM-4662-MT-1.2	Mono-(2-ethyl-5-hydroxyhexyl) phthalate (DEHP Metabolite IX) (unlabeled) 100 µg/mL in MTBE
CLM-6640-MT-1.2	Mono-(2-ethyl-5-oxohexyl) phthalate (DEHP Metabolite VI) ($^{13}C_4$, 99%) 100 µg/mL in MTBE
ULM-4663-MT-1.2	Mono-(2-ethyl-5-oxohexyl) phthalate (DEHP Metabolite VI) (unlabeled) 100 µg/mL in MTBE
CLM-4586-MT-1.2	Monoethyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-4585-MT-1.2	Monoethyl phthalate (unlabeled) 100 µg/mL in MTBE
ULM-7919-MT-1.2	Monoisobutyl phthalate (unlabeled) 100 µg/mL in MTBE
ULM-4652-1.2	Monoisodecyl phthalate (Mono-3,7-dimethyloctyl phthalate) (unlabeled) 100 µg/mL in acetonitrile
CLM-4587-MT-1.2	Monoisononyl phthalate (Mono-3,5,5-trimethylhexyl phthalate) (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-4651-MT-1.2	Monoisononyl phthalate (Mono-3,5,5-trimethylhexyl phthalate) (unlabeled) 100 µg/mL in MTBE
ULM-7395-1.2	Monoisopropyl phthalate (unlabeled) 100 µg/mL in acetonitrile
CLM-6071-1.2	Monomethyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in acetonitrile
ULM-6697-MT-1.2	Monomethyl phthalate (unlabeled) 100 µg/mL in MTBE
CLM-4589-MT-1.2	Mono-*n*-octyl phthalate (ring-1,2-$^{13}C_2$, dicarboxyl-$^{13}C_2$, 99%) 100 µg/mL in MTBE
ULM-4593-MT-1.2	Mono-*n*-octyl phthalate (unlabeled) 100 µg/mL in MTBE
ULM-7393-1.2	Mono-*n*-pentyl phthalate (unlabeled) 100 µg/mL in acetonitrile

Prescription and Non-Prescription Drug Standards

Catalog No.	Description
CNLM-3726-1.2	Acetaminophen (acetyl-$^{13}C_2$, 99%;^{15}N, 98%) 100 µg/mL in acetonitrile
ULM-7629-1.2	Acetaminophen (unlabeled) 100 µg/mL in acetonitrile
DLM-3008-1.2	Amitriptyline•HCl (*N,N*-dimethyl-D_6, 98%) 100 µg/mL in methanol
ULM-8350-1.2	Amitriptyline•HCl (unlabeled) 100 µg/mL in methanol
CLM-514-1.2	Caffeine (trimethyl-$^{13}C_3$, 99%) 100 µg/mL in methanol
ULM-7653-1.2	Caffeine (unlabeled) 100 µg/mL in methanol
DLM-2806-1.2	Carbamazepine (D_{10}, 98%) 100 µg/mL in acetonitrile-D_3
ULM-6581-1.2	Carbamazepine (unlabeled) CP 97% 100 µg/mL in acetonitrile
DLM-1287-1.2	Clonidine (4,4,5,5-imidazoline-D_4, 98%) 100 µg/mL in methanol
ULM-8349-1.2	Clonidine (unlabeled) 100 µg/mL in methanol
C-041	Codeine (D_6, 98%) 1.0 mg/mL in methanol
C-006	Codeine (unlabeled) 1.0 mg/mL in methanol
C-035	(+/-)-Cotinine (D_3, 98%) 1.0 mg/mL in methanol
C-016	(-)-Cotinine (unlabeled) 1.0 mg/mL in methanol
D-902	Diazepam (D_5, 98%) 100 µg/mL in methanol
D-907	Diazepam (unlabeled) 1.0 mg/mL in methanol
DLM-8567-1.2	Diclofenac (phenyl-D_4, 98%) 100 µg/mL in methylene chloride CP 96%
ULM-9023-1.2	Diclofenac (unlabeled) 100 µg/mL in methylene chloride
CNLM-411-1.2	5,5-Diphenylhydantoin (2-^{13}C, 99%;1,3-$^{15}N_2$, 98%) 100 µg/mL in methanol
ULM-8533-1.2	5,5-Diphenylhydantoin (unlabeled) 100 µg/mL in methanol

Catalog No.	Description
F-919	Fluoxetine oxalate (D_6, 98%) 100 µg/mL in methanol
F-918	Fluoxetine•HCl (unlabeled) 1.0 mg/mL in methanol
DLM-8221-1.2	Gemfibrozil (2,2-dimethyl-D_6, 98%) 100 µg/mL in *p*-dioxane
ULM-8225-1.2	Gemfibrozil (unlabeled) 100 µg/mL in *p*-dioxane
CLM-6943-1.2	Ibuprofen (propionic-$^{13}C_3$, 99%) 100 µg/mL in acetonitrile
ULM-7275-1.2	Ibuprofen (unlabeled) 100 µg/mL in acetonitrile
I-902	Imipramine (unlabeled) 1.0 mg/mL in methanol
L-902	Lorazepam (D_4, 98%) 100 µg/mL in acetonitrile
L-901	Lorazepam (unlabeled) 1.0 mg/mL in acetonitrile
CDLM-7665-1.2	Naproxen (methyl-^{13}C, 99% methyl-D_3, 98%) 100 µg/mL in acetonitrile
ULM-7709-1.2	Naproxen (unlabeled) 100 µg/mL in acetonitrile
N-922	Norfluoxetine oxalate (D_6, 98%) 100 µg/mL in methanol
N-923	Norfluoxetine oxalate (unlabeled) 1.0 mg/mL in methanol
DLM-3039-1MG	Phenylbutazone (diphenyl-D_{10}, 98%) neat
ULM-7378-1MG	Phenylbutazone (unlabeled) neat
CLM-7892	Resorcinol ($^{13}C_6$, 99%)
CLM-8370-1.2	Thiabendazole (ring-$^{13}C_6$, 99%) 100 µg/mL in acetonitrile
ULM-8371-1.2	Thiabendazole (unlabeled) 100 µg/mL in acetonitrile
DLM-6861-1.2	Warfarin (phenyl-D_5, 98%) 100 µg/mL in acetonitrile-D_3
ULM-7242-1.2	Warfarin (unlabeled) 100 µg/mL in acetonitrile

RNA/DNA

Catalog No.	Description
CLM-1654	Adenine (8-^{13}C, 95%)
NLM-6924	Adenine•HCl ($^{15}N_5$, 98%)
CLM-3678	Adenosine (ribose-$^{13}C_5$, 98%+) CP 97%
NLM-3796	Adenosine•H_2O ($^{15}N_5$, 96-98%)
CNLM-3806-CA	Adenosine ($^{13}C_{10}$, 98%; $^{15}N_5$, 96-98%)
CNLM-4265-CA	Adenosine 5′-triphosphate, ammonium salt (^{13}C; ^{15}N, 98-99%) CP >90% (in solution)
NLM-3987-CA	Adenosine 5′-triphosphate, ammonium salt ($^{15}N_5$, 98-99%) CP >90% (in solution)
NLM-3797	Cytidine ($^{15}N_3$, 96-98%)
CNLM-3807	Cytidine ($^{13}C_9$, 98%; $^{15}N_3$, 96-98%)
NLM-4266-CA	Cytidine 5′-triphosphate, ammonium salt ($^{15}N_3$, 96%) CP >90% (in solution)
CNLM-4267-CA	Cytidine 5′-triphosphate, ammonium salt (^{13}C; ^{15}N, 96-98%) CP >90% (in solution)
NLM-3895	2′-Deoxyadenosine ($^{15}N_5$, 96-98%)
CNLM-3896	2′-Deoxyadenosine ($^{13}C_{10}$, 98%; $^{15}N_5$, 96-98%)
NLM-7659	2′-Deoxyadenosine phosphoramidite ($^{15}N_5$, 98%) (dibenzoate)
CNLM-6828	2′-Deoxyadenosine phosphoramidite ($^{13}C_{10}$, 98%; $^{15}N_5$, 98%) CP 95%
CNLM-6219-CA	2′-Deoxyadenosine 5′-triphosphate ($^{13}C_{10}$, 98%; $^{15}N_5$, 97-98%) CP >90%
NLM-3897	2′-Deoxycytidine ($^{15}N_3$, 96-98%)
NLM-3921	2′-Deoxycytidine 5′-monophosphate ($^{15}N_3$, 96%)
NLM-6827	2′-Deoxycytidine phosphoramidite ($^{15}N_3$, 98%) CP 95%
CNLM-6830	2′-Deoxycytidine phosphoramidite ($^{13}C_9$, 98%; $^{15}N_3$, 98%) CP 95%
NLM-3899-CA	2′-Deoxyguanosine•H_2O ($^{15}N_5$, 98%) CP 95%+
NLM-6826	2′-Deoxyguanosine phosphoramidite (U-$^{15}N_5$, 98%) CP 95%
CNLM-6825	2′-Deoxyguanosine phosphoramidite (U-$^{13}C_{10}$, 98%; U-$^{15}N_5$, 98%) CP 95%
NLM-6217-CA	2′-Deoxyguanosine 5′-triphosphate, ammonium salt (U-$^{15}N_5$; 98-99%) CP >90% (in solution)
CNLM-6221-CA	2′-Deoxyguanosine 5′-triphosphate, ammonium salt (U-^{13}C, 98%; U-^{15}N, 96-98%) CP >90%
CNLM-8771-CA	2′-Deoxyuridine•H_2O ($^{13}C_9$, 98-99%; $^{15}N_2$, 98-99%) CP 90% (in solution)

Catalog No.	Description
CNLM-4510	5,6-Dihydrouracil ($^{13}C_4$, 99%; $^{15}N_2$, 98%+)
DLM-4391	5,6-Dihydrothymine (5,6,6-D_3, methyl-D_3, 95%+)
CNLM-3916	5-Fluorouracil ($^{13}C_4$, 99%; $^{15}N_2$, 98%)
NLM-6925	Guanine ($^{15}N_5$, 98%)
NLM-3798	Guanosine (U-$^{15}N_5$, 96-98%)
NLM-4268-CA	Guanosine 5′-triphosphate, ammonium salt (U-$^{15}N_5$, 98-99%) CP >90% (in solution)
CNLM-4269-CA	Guanosine 5′-triphosphate, ammonium salt (U-^{13}C; U-^{15}N, 98-99%); CP >90% (in solution)
CNLM-3808-CA	Guanosine•H_2O (U-$^{13}C_{10}$, 98%; U-$^{15}N_5$, 96-98%)
CNLM-3859	8-Hydroxyguanine•1/2 H_2O (8-^{13}C, 98%; 7,9-$^{15}N_2$, 98%)
NLM-4264	Inosine (U-$^{15}N_4$, 95%+)
NLM-8712-CA	Inosine 5′-monophosphate, ammonium salt (U-$^{15}N_4$, 98-99%) CP >90% (in solution)
DLM-7471	3-Methyladenine (methyl-D_3, 98%)
DLM-7472	7-Methylguanine (methyl-D_3, 98%)
CLM-3647	Thymidine (methyl-^{13}C, 98%)
NLM-3901	Thymidine (U-$^{15}N_2$, 96-98%) CP 97%
CNLM-3902	Thymidine (U-$^{13}C_{10}$, 98%; U-$^{15}N_2$, 96-98%)
NLM-3925	Thymidine 5′-monophosphate (U-$^{15}N_2$, 98%)
NLM-6823	Thymidine phosphoramidite (U-$^{15}N_2$, 96-98%) CP 95%
CNLM-6824	Thymidine phosphoramidite (U-$^{13}C_{10}$, 98%; U-$^{15}N_2$, 98%) CP 95%
CNLM-6945	Thymine (U-$^{13}C_5$, 98%; U-$^{15}N_2$, 98%)
NLM-3795	Uridine 5′-monophosphate (U-$^{15}N_2$, 96-98%)
CNLM-3805	Uridine 5′-monophosphate (U-$^{13}C_9$, 98%; U-$^{15}N_2$, 96-98%)
DLM-7517-CA	Uridine 5′-triphosphate, ammonium salt (D_8, 97%+) CP 90%+
DLM-8925-CA	Uridine 5′-triphosphate (UTP), ammonium salt (5-D_1, ribose-3′,4′,5′,5″-D_4, 98%) CP >90% (in solution)
NLM-4270-CA	Uridine 5′-triphosphate, ammonium salt (U-$^{15}N_2$, 98-99%) CP >90% (in solution)
CNLM-4271-CA	Uridine 5′-triphosphate, ammonium salt (U-^{13}C; U-^{15}N, 98-99%) CP >90% (in solution)
CLM-8700-CA	Xanthosine-5′-monophosphate, ammonium salt (U-$^{13}C_{10}$, 98%) CP >90% (in solution)

^{15}N Salts

Catalog No.	Description
NLM-467	Ammonium chloride (^{15}N, 99%)
NLM-711	Ammonium nitrate (ammonium-^{15}N, 98%+)
NLM-712	Ammonium nitrate (nitrate-^{15}N, 98%+)
NLM-390	Ammonium nitrate ($^{15}N_2$, 98%+)
NLM-713	Ammonium sulfate ($^{15}N_2$, 99%)
NLM-499	Calcium nitrate ($^{15}N_2$, 98%+)
NLM-765	Potassium nitrate (^{15}N, 99%)
NLM-157	Sodium nitrate (^{15}N, 98%+)

SILAC Kits and Reagents

SILAC Protein Quantitation Kits

Catalog No.	Description
DMEM-LYS-C	SILAC Protein Quantitation Kit DMEM (Dulbecco's Modified Eagle Media)
RPMI-LYS-C	SILAC Protein Quantitation Kit RPMI 1640
DMEM-500	DMEM Media for SILAC (DMEM minus L-Lysine and L-Arginine)
RPMI-500	RPMI 1640 Media for SILAC (RPMI 1640 minus L-Lysine and L-Arginine)
FBS-50	Dialyzed Fetal Bovine Serum

Arginine

Catalog No.	Description
CLM-1268	L-Arginine•HCl (1-^{13}C, 99%)
CLM-2070	L-Arginine•HCl (guanido-^{13}C, 99%)
CLM-2051	L-Arginine•HCl (1,2-$^{13}C_2$, 99%)
CLM-2265-H	L-Arginine•HCl ($^{13}C_6$, 99%)
DLM-6038	L-Arginine•HCl (<5% D) (4,4,5,5-D_4, 94%)
DLM-541	L-Arginine•HCl (D_7, 98%)
NLM-1267	L-Arginine•HCl (α-^{15}N, 98%+)
NLM-395	L-Arginine•HCl (guanido-$^{15}N_2$, 98%+)
NLM-396	L-Arginine•HCl ($^{15}N_4$, 98%)
CNLM-7819	L-Arginine•HCl (1-^{13}C, 99%; α-^{15}N, 98%)
CNLM-539-H	L-Arginine•HCl ($^{13}C_6$, 99%; $^{15}N_4$, 99%)
CDLM-3789	L-Arginine•HCl (5-^{13}C, 99%; 4,4,5,5-D_4, 95%)
DNLM-7543	L-Arginine•HCl (D_7, 98%; $^{15}N_4$, 98%)
CDNLM-6801	L-Arginine•HCl ($^{13}C_6$, 97-99%; D_7, 97-99%; $^{15}N_4$, 97-99%)
ULM-8347	L-Arginine•HCl (unlabeled)

Leucine

Catalog No.	Description
CLM-2262-H	L-Leucine ($^{13}C_6$, 99%)
DLM-4212	L-Leucine (isopropyl-D_7, 98%)
CNLM-281-H	L-Leucine ($^{13}C_6$, 99%; ^{15}N, 99%)
CDNLM-4280	L-Leucine ($^{13}C_6$, 95-97%; ^{15}N, 96-99%; 2,3,3-D_3, 97%+)

Lysine

Catalog No.	Description
CLM-653	L-Lysine•2HCl (1-^{13}C, 99%)
CLM-633	L-Lysine•HCl (5-^{13}C, 99%)
CLM-632	L-Lysine•2HCl (6-^{13}C, 99%)
CLM-2247-H	L-Lysine•2HCl ($^{13}C_6$, 99%)
DLM-2640	L-Lysine•2HCl (4,4,5,5-D_4, 96-98%)
DLM-2641	L-Lysine•2HCl (3,3,4,4,5,5,6,6-D_8, 98%)
DLM-570	L-Lysine•2HCl (D_9, 98%)
NLM-143	L-Lysine•2HCl (α-^{15}N, 95-99%)
NLM-631	L-Lysine•2HCl (ε-^{15}N, 98%+)
NLM-1554	L-Lysine•2HCl ($^{15}N_2$, 98%+)
CNLM-7821	L-Lysine•2HCl (1-^{13}C, 99%; α-^{15}N, 98%)
CNLM-3454	L-Lysine•2HCl•H_2O (6-^{13}C, 99%; ε-^{15}N, 98%)
CNLM-291-H	L-Lysine•2HCl ($^{13}C_6$, 99%; $^{15}N_2$, 99%)
DNLM-7545	L-Lysine•2HCl (D_9, 98%; $^{15}N_2$, 98%)
CDNLM-6810	L-Lysine•2HCl ($^{13}C_6$, 97-99%; D_9, 97-99%; $^{15}N_2$, 97-99%)
ULM-8766	L-Lysine•2HCl (unlabeled)

Please see pages 130-136 for a complete listing of amino acids.

Spirulina

Spirulina Whole Cells

Catalog No.	Description
CLM-8400	Spirulina whole cells (lyophilized powder) (U-^{13}C, 97%+)
NLM-8401	Spirulina whole cells (lyophilized powder) (U-^{15}N, 98%+)
ULM-8453	Spirulina whole cells (lyophilized powder) unlabeled

Please see page 148 for MouseExpress® (^{15}N, 98%) Mouse Feed.

Steroids

Catalog No.	Description
DLM-8438	Aldosterone (2,2,4,6,6,17,21,21-D_8)
ULM-9134	Aldosterone (unlabeled) CP 95%
DLM-8750	5β-Androstan-3α-ol-17-one (16,16-D_2, 98%)
CLM-9135	4-Androstene-3,17-dione (2,3,4-$^{13}C_3$, 99%)
CLM-9135-C	4-Androstene-3,17-dione (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in 1,2-dimethoxyethane
CLM-9135-D	4-Androstene-3,17-dione (2,3,4-$^{13}C_3$, 99%) 1000 µg/mL in 1,2-dimethoxyethane
DLM-7976	4-Androstene-3,17-dione (2,2,4,6,6,16,16-D_7, 97%)
ULM-8472	4-Androstene-3,17-dione (unlabeled)
ULM-8472-C	4-Androstene-3,17-dione (unlabeled) 100 µg/mL in 1,2-dimethoxyethane
ULM-8472-D	4-Androstene-3,17-dione (unlabeled) 1000 µg/mL in 1,2-dimethoxyethane
DLM-7937	Androsterone (16,16-D_2, 98%)
DLM-9137	Androsterone glucuronide (2,2,4,4-D_4, 98%)
ULM-9138	Androsterone glucuronide (unlabeled)
DLM-6780	Chenodeoxycholic acid (2,2,4,4-D_4, 98%)
DLM-4700	Cholestane (3,3-D_2, 98%)
DLM-8276	Cholestenone (2,2,4,6,6-D_5, 98%)
CLM-9139	Cholesterol (2,3,4-$^{13}C_3$, 99%)
CLM-9139-B	Cholesterol (2,3,4-$^{13}C_3$, 99%) 50 µg/mL in chloroform
CLM-9139-C	Cholesterol (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in chloroform
CLM-804	Cholesterol (3,4-$^{13}C_2$, 99%)
DLM-1831	Cholesterol (3-D_1, 98%)
DLM-7260	Cholesterol (25,26,26,26-D_4, 98%)
DLM-2607	Cholesterol (2,2,3,4,4,6-D_6, 97-98%)
DLM-3057	Cholesterol (25,26,26,26,27,27,27-D_7, 98%)
OLM-7695	Cholesterol (^{18}O, 80%)
ULM-9140	Cholesterol (unlabeled)
ULM-9140-C	Cholesterol (unlabeled) 100 µg/mL in chloroform
ULM-9140-D	Cholesterol (unlabeled) 1000 µg/mL in chloroform
CLM-3361	Cholesterol-3-octanoate (octanoate-1-^{13}C, 99%)
CLM-2710	Cholic acid (24-^{13}C, 99%)
DLM-2611	Cholic acid (2,2,4,4-D_4, 98%)
DLM-7347	Corticosterone (2,2,4,6,6,17a,21,21-D_8, 97-98%)
DLM-2615	Cortisol (1,2-D_2, 98%)
DLM-2057	Cortisol (9,12,12-D_3, 98%)
DLM-2218	Cortisol (9,11,12,12-D_4, 98%)
ULM-9141	Cortisol (unlabeled)
ULM-9141-C	Cortisol (unlabeled) 100 µg/mL in methanol
ULM-9141-D	Cortisol (unlabeled) 1000 µg/mL in methanol
ULM-7823	Cortisol (unlabeled)
DLM-8863	Cortisone (1,2-D_2, 98%)
DLM-9142	Cortisone (2,2,4,6,6,12,12-D_7, 98%)
ULM-9202	Cortisone (unlabeled)
DLM-4216	7-Dehydrocholesterol (25,26,26,26,27,27,27-D_7, 98%)
DLM-7714	Dehydroepiandrosterone (DHEA) (16,16-D_2, 97%)
ULM-9143	Dehydroepiandrosterone (DHEA) (unlabeled)
ULM-9143-C	Dehydroepiandrosterone (DHEA) (unlabeled) 100 µg/mL in methanol
ULM-9143-D	Dehydroepiandrosterone (DHEA) (unlabeled) 1000 µg/mL in methanol

Catalog No.	Description
DLM-8701	Dehydroepiandrosterone sulfate•sodium salt (DHEAS) (16,16-D_2, 97%)
DLM-8337	Dehydroepiandrosterone sulfate•sodium salt (DHEAS) (2,2,3,4,4,6-D_6, 98%)
ULM-9144	Dehydroepiandrosterone sulfate•sodium salt (DHEAS) (unlabeled)
ULM-9144-C	Dehydroepiandrosterone sulfate•sodium salt (DHEAS) (unlabeled) 100 µg/mL in methanol
ULM-9144-D	Dehydroepiandrosterone sulfate•sodium salt (DHEAS) (unlabeled) 1000 µg/mL in methanol
CLM-3364	Deoxycholic acid (24-^{13}C, 99%)
DLM-2824	Deoxycholic acid (2,2,4,4-D_4, 98%)
DLM-7209	11-Deoxycortisol (21,21-D_2, 96%)
ULM-9145	11-Deoxycortisol (unlabeled)
ULM-9145-C	11-Deoxycortisol (unlabeled) 100 µg/mL in methanol
ULM-9145-D	11-Deoxycortisol (unlabeled) 1000 µg/mL in methanol
DLM-8305	21-Deoxycortisol (D_8, 96%)
DLM-170	Diethylstilbestrol (*cis*/*trans* mix) (ring-3,3′,5,5′-diethyl-1,1,1′,1′-D_8, 98%)
ULM-7921	Diethylstilbestrol (*cis*/*trans* mix) (unlabeled)
DLM-3023	Dihydrotestosterone (16,16,17-D_3, 98%)
CLM-9146	5α-Dihydrotestosterone (2,3,4-$^{13}C_3$, 99%) CP 97%
CLM-9146-C	5α-Dihydrotestosterone (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in 1,2-dimethoxyethane
CLM-9146-D	5α-Dihydrotestosterone (2,3,4-$^{13}C_3$, 99%) 1000 µg/mL in 1,2-dimethoxyethane
DLM-9041	5α-Dihydrotestosterone (2,2,4,4-D_4, 98%)
CNLM-7889	DL-Epinephrine (1,2-$^{13}C_2$, 99%; ^{15}N, 98%)
DLM-2866	DL-Epinephrine (α,α,β-D_3, 97%)
CLM-7936	Estradiol (13,14,15,16,17,18-$^{13}C_6$, 99%) 100 µg/mL in methanol
DLM-3694	Estradiol (16,16,17-D_3, 98%)
DLM-2487	Estradiol (2,4,16,16-D_4, 95-97%)
ULM-7449	Estradiol (unlabeled) 100 µg/mL in nonane
CLM-9147	Estriol (16α-hydroxyestradiol) (2,3,4-$^{13}C_3$, 99%)
CLM-9147-A	Estriol (16α-hydroxyestradiol) (2,3,4-$^{13}C_3$, 99%) 5 µg/mL in methanol
CLM-9147-B	Estriol (16α-hydroxyestradiol) (2,3,4-$^{13}C_3$, 99%) 50 µg/mL in methanol
CLM-9147-C	Estriol (16α-hydroxyestradiol) (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in methanol
DLM-7468	Estriol (2,4-D_2, 98%)
DLM-8343	Estriol (2,4,17-D_3, 98%) CP 96%
DLM-8583	Estriol (2,4,16,17-D_4, 98%) CP 95%
DLM-8586	Estriol (2,4,16-D_3, 98%)
ULM-8218	Estriol (unlabeled)
CLM-9148	Estrone (2,3,4-$^{13}C_3$, 99%)
CLM-9148-B	Estrone (2,3,4-$^{13}C_3$, 99%) 50 µg/mL in methanol
CLM-9148-C	Estrone (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in methanol
CLM-673	Estrone (3,4-$^{13}C_2$, 99%) 100 µg/mL in acetonitrile
DLM-3976	Estrone (2,4,16,16-D_4, 97%)
CLM-8033	DL-Estrone 3-methyl ether (13,14,15,16,17,18-$^{13}C_6$, 99%)
CLM-3375	Ethynylestradiol (20,21-$^{13}C_2$, 99%) 100 µg/mL in acetonitrile
DLM-4691	17-α-Ethynylestradiol (2,4,16,16-D_4, 97-98%)
ULM-7211	Ethynylestradiol (unlabeled) 100 µg/mL in acetonitrile

CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

Steroids

Catalog No.	Description
DLM-8646	7-β-Hydroxycholesterol (25,26,26,26,27,27,27-D_7, 98%) CP 97%
DLM-9150	18-Hydroxycorticosterone (9,11,12,12-D_4, 98%) CP 95%
ULM-9151	18-Hydroxycorticosterone (unlabeled) CP 95%
DLM-9149	6β-Hydroxycortisol (9,11,12,12-D_4) CP 97%
CLM-8012	DL-2-Hydroxyestradiol (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8135	2-Hydroxyestradiol (unlabeled)
ULM-8134	2-Hydroxyestrone (unlabeled)
CLM-8013	DL-4-Hydroxyestrone (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8261	4-Hydroxyestrone (unlabeled) CP 96%
CLM-9153	16α-Hydroxyestrone (2,3,4-$^{13}C_3$, 99%)
ULM-9152	16α-Hydroxyestrone (unlabeled)
CLM-8016	DL-2-Hydroxyestrone-3-methyl ether (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8133	2-Hydroxyestrone-3-methyl ether (unlabeled)
DLM-7206	17-Hydroxypregnenolone (21,21,21-D_3, 97%)
CDLM-9154	17α-Hydroxypregnenolone (20,21-$^{13}C_2$, 99%; 16,16-D_2, 99%)
CDLM-9154-C	17α-Hydroxypregnenolone (20,21-$^{13}C_2$, 99%; 16,16-D_2, 99%) 100 µg/mL in methanol
CDLM-9154-D	17α-Hydroxypregnenolone (20,21-$^{13}C_2$, 99%; 16,16-D_2, 99%) 1000 µg/mL in methanol
ULM-9155	17α-Hydroxypregnenolone (unlabeled)
ULM-9155-C	17α-Hydroxypregnenolone (unlabeled) 100 µg/mL in methanol
ULM-9155-D	17α-Hydroxypregnenolone (unlabeled) 1000 µg/mL in methanol
CLM-9157	17α-Hydroxyprogesterone (2,3,4-$^{13}C_3$, 98%)
CLM-9157-C	17α-Hydroxyprogesterone (2,3,4-$^{13}C_3$, 98%) 100 µg/mL in methanol
CLM-9157-D	17α-Hydroxyprogesterone (2,3,4-$^{13}C_3$, 98%) 1000 µg/mL in methanol
DLM-6598	17-Hydroxyprogesterone (2,2,4,6,6,21,21,21-D_8, 98%)
ULM-9156	17α-Hydroxyprogesterone (unlabeled)
ULM-9156-C	17α-Hydroxyprogesterone (unlabeled) 100 µg/mL in methanol CP 95%
ULM-9156-D	17α-Hydroxyprogesterone (unlabeled) 1000 µg/mL in methanol CP 95%
DLM-8647	7-Ketocholesterol (25,26,26,26,27,27,27-D_7, 99%)
DLM-3560	DL-Metanephrine•HCl (α,β,β-D_3, 98%)
CLM-8015	DL-2-Methoxyestradiol (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8137	2-Methoxyestradiol (unlabeled)
CLM-8014	DL-2-Methoxyestrone (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8263	2-Methoxyestrone (unlabeled)
CLM-8017	DL-4-Methoxyestrone (13,14,15,16,17,18-$^{13}C_6$, 99%)
ULM-8262	4-Methoxyestrone (unlabeled)
CLM-2468	Norethindrone (ethynyl-$^{13}C_2$, 99%)
DLM-3670	DL-Norepinephrine•HCl (1,2,2-D_3, 95%)
DLM-8820	DL-Norepinephrine•HCl (ring-D_3,1,2,2-D_3, 99%)
DLM-3979	19-Nortestosterone (16,16,17-D_3, 98%)
ULM-4841	19-Nortestosterone (unlabeled)
ULM-222	Pregna-1,4,6-triene-3,20-dione (unlabeled)
DLM-3754	5-α-Pregnan-3-α-ol-20-one (17,21,21,21-D_4, 96-98%) CP 95%+
ULM-3779	5-α-Pregnan-3-α-ol-20-one (unlabeled) CP 97%
DLM-7492	5-α-Pregnan-3-β-ol-20-one (17α,21,21,21-D_4, 97%+) CP 96%
ULM-8242	5-α-Pregnan-3-β-ol-20-one (unlabeled)
DLM-2294	5-β-Pregnan-3-α-ol-20-one (17,21,21,21-D_4, 96-98%)

Catalog No.	Description
DLM-8751	5-β-Pregnan-3-α,11-β,17-α,21-tetrol-20-one (9,11α,12-D_3, 95%)
DLM-8753	5-β-Pregnan-3-α,17-α,20-triol (20,21,21,21-D_4, 98%) mix of 20α & 20β
DLM-3910	5-α-Pregnane-3-α,21-diol-20-one (17,21,21-D_3, 95%)
DLM-3816	5-α-Pregnane-3,20-dione (1,2,4,5,6,7-D_6, 95%)
DLM-3817	5-β-Pregnane-3,20-dione (1,2,4,5,6,7-D_6, 95%)
DLM-7228	4-Pregnen-21-ol-3,20-dione (2,2,4,6,6,17,21,21-D_8, 96%) CP 95%
DLM-6896	Pregnenolone (17,21,21,21-D_4, 98%)
CDLM-9158	Pregnenolone (20,21-$^{13}C_2$, 99%;16,16-D_2, 98%)
ULM-9159	Pregnenolone (unlabeled)
CDLM-9160	Pregnenolone sulfate•sodium salt (20,21-$^{13}C_2$, 99%;16,16-D_2, 98%)
ULM-9161	Pregnenolone sulfate•sodium salt (unlabeled)
CLM-457	Progesterone (3,4-$^{13}C_2$, 90%)
CLM-9162	Progesterone (2,3,4-$^{13}C_3$, 99%)
CLM-9162-B	Progesterone (2,3,4-$^{13}C_3$, 99%) 50 µg/mL in acetonitrile
CLM-9162-C	Progesterone (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in acetonitrile
DLM-6909	Progesterone (2,2,6,6,17,21,21,21-D_8, 96%)
DLM-7953	Progesterone (2,2,4,6,6,17α,21,21,21-D_9, 98%)
ULM-8219	Progesterone (unlabeled)
DLM-3312	Prostaglandin A2 (3,3,4,4-D_4, 98%)
DLM-3627	Prostaglandin A2 (3,3,4,4-D_4, 98%) (in solution)
DLM-3728	Prostaglandin E1 (3,3,4,4-D_4, 98%) (in solution)
DLM-3592	Prostaglandin E2 (3,3,4,4-D_4, 98%) 500 µg/mL in methyl acetate
DLM-3628	Prostaglandin E2 (3,3,4,4-D_4, 98%) (in solution)
DLM-3558	Prostaglandin F2α (3,3,4,4-D_4, 98%) (in solution)
DLM-4200	9-α,11-α-Prostaglandin F2 (3,3',4,4'-D_4, 98%) (in solution)
DLM-7457	Sodium 17β-estradiol 3-sulfate (2,4,16,16-D_4, 98%) (stabilized with 50% w/w tris)
DLM-7456	Sodium estrone 3-sulfate (2,4,16,16-D_4, 98%) (stabilized with 50% w/w tris)
CLM-159	Testosterone (3,4-$^{13}C_2$, 99%)
CLM-9164	Testosterone (2,3,4-$^{13}C_3$, 99%)
CLM-9164-C	Testosterone (2,3,4-$^{13}C_3$, 99%) 100 µg/mL in 1,2-dimethoxyethane
CLM-9164-D	Testosterone (2,3,4-$^{13}C_3$, 99%) 1000 µg/mL in 1,2-dimethoxyethane
DLM-683	Testosterone (1,2-D_2, 98%)
DLM-6224	Testosterone (16,16,17-D_3, 98%)
DLM-8085	Testosterone (2,2,4,6,6-D_5, 98%)
ULM-8081	Testosterone (unlabeled)
ULM-8933	Testosterone benzoate (unlabeled)
DLM-8265	Testosterone diacetate (testosterone-D_4, acetate methyl-D_6, 98%)
ULM-9163	3α,5β-Tetrahydroaldosterone (unlabeled)
DLM-7477	3-α,5-β-Tetrahydrodeoxycorticosterone (17,21,21-D_3, 97%) CP 96%
CLM-7185	3,3',5-Triiodo-L-thyronine (ring-$^{13}C_6$, 99%) CP >90%
CLM-6725	L-Thyroxine (tyrosine-ring-$^{13}C_6$, 99%) CP 90%
CLM-8931	L-Thyroxine (ring-$^{13}C_{12}$, 99%) CP 97%
ULM-8184	L-Thyroxine (unlabeled)

CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

Veterinary and Human Antibiotic Standards

Catalog No.	Description
DLM-7170-1.2	1-Aminohydantoin hydrochloride (AHD) (5,5-D_2, 98%) 100 µg/mL in acetonitrile-D_3
ULM-7188-1.2	1-Aminohydantoin hydrochloride (AHD) (unlabeled) 100 µg/mL in methanol
DLM-7171-1.2	3-Amino-2-oxazolidone (AOZ) (ring-D_4, 98%) 100 µg/mL in acetonitrile-D_3
ULM-7189-1.2	3-Amino-2-oxazolidone (AOZ) (unlabeled) 100 µg/mL in methanol
CLM-7407-1MG	Amoxicillin•3H_2O (phenyl-$^{13}C_6$, 99%) neat
DLM-119-1.2	(+/-)-Chloramphenicol (ring-D_4, benzyl-D_1, 98%) 100 µg/mL in acetonitrile
ULM-6687-1.2	(+/-)-Chloramphenicol (unlabeled) 100 µg/mL in acetonitrile
CNLM-7539-1.2	Ciprofloxacin•HCl (2,3,carboxyl-$^{13}C_3$, 99%; quinoline-^{15}N, 98%) 100 µg/mL in methanol
ULM-7710-1.2	Ciprofloxacin•HCl (unlabeled) 100 µg/mL in methanol

Catalog No.	Description
CLM-3672-1.2	Erythromycin (90-95% Erythromycin A) (*N,N*-dimethyl-$^{13}C_2$, ~90%) 100 µg/mL in acetonitrile
ULM-4322-1.2	Erythromycin (unlabeled) 100 µg/mL in acetonitrile
DLM-7172-1.2	5-(4-Morpholinylmethyl)-3-amino-2-oxazolidinone (AMOZ) (4,4,5,5′,5′-D_5, 98%) 100 µg/mL in acetonitrile-D_3
ULM-7190-1.2	5-(4-Morpholinylmethyl)-3-amino-2-oxazolidinone (AMOZ) (unlabeled) 100 µg/mL in methanol
CLM-3045-1.2	Sulfamethazine (phenyl-$^{13}C_6$, 90%) 100 µg/mL in acetonitrile
ULM-7220-1.2	Sulfamethazine (unlabeled) 100 µg/mL in acetonitrile
CLM-6944-1.2	Sulfamethoxazole (ring-$^{13}C_6$, 99%) 100 µg/mL in acetonitrile
ULM-7527-1.2	Sulfamethoxazole (unlabeled) 100 µg/mL in acetonitrile
CLM-7988-A-1.2	Trimethoprim (pyrimidine-4,5,6-$^{13}C_3$, 99%) 50 µg/mL in methanol
ULM-7989-A-1.2	Trimethoprim (unlabeled) 50 µg/mL in methanol

CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

Vitamins

Catalog No.	Description
CLM-3085	L-Ascorbic acid (1-^{13}C, 99%)
CLM-7283	L-Ascorbic acid (U-$^{13}C_6$, 98%)
DLM-8806	Biotin (ring-6,6-D_2, 98%) CP 97%
DLM-9105	1,25-Dihydroxyvitamin D_2 (6,19,19-D_3, 99%) CP 95%
DLM-9105-A	1,25-Dihydroxyvitamin D_2 (6,19,19-D_3, 99%) 5 µg/mL in ethanol CP 95%
DLM-9105-B	1,25-Dihydroxyvitamin D_2 (6,19,19-D_3, 99%) 50 µg/mL in ethanol CP 95%
DLM-9105-C	1,25-Dihydroxyvitamin D_2 (6,19,19-D_3, 99%) 100 µg/mL in ethanol CP 95%
ULM-9106	1,25-Dihydroxyvitamin D_2 (unlabeled) CP 95%
ULM-9106-A	1,25-Dihydroxyvitamin D_2 (unlabeled) 5 µg/mL in ethanol CP 95%
ULM-9106-B	1,25-Dihydroxyvitamin D_2 (unlabeled) 50 µg/mL in ethanol CP 95%
ULM-9106-C	1,25-Dihydroxyvitamin D_2 (unlabeled) 100 µg/mL in ethanol CP 95%
ULM-9109	24R,25-Dihydroxyvitamin D_2 (unlabeled)
ULM-9109-B	24R,25-Dihydroxyvitamin D_2 (unlabeled) 50 µg/mL in ethanol
ULM-9109-C	24R,25-Dihydroxyvitamin D_2 (unlabeled) 100 µg/mL in ethanol
DLM-9107	1,25-Dihydroxyvitamin D_3 (6,19,19-D_3, 97%) CP 95%
DLM-9107-A	1,25-Dihydroxyvitamin D_3 (6,19,19-D_3, 97%) 5 µg/mL in ethanol CP 95%
DLM-9107-B	1,25-Dihydroxyvitamin D_3 (6,19,19-D_3, 97%) 50 µg/mL in ethanol CP 95%
DLM-9107-C	1,25-Dihydroxyvitamin D_3 (6,19,19-D_3, 97%) 100 µg/mL in ethanol CP 95%
ULM-9108	1,25-Dihydroxyvitamin D_3 (unlabeled) CP 95%
ULM-9108-A	1,25-Dihydroxyvitamin D_3 (unlabeled) 5 µg/mL in ethanol CP 95%
ULM-9108-B	1,25-Dihydroxyvitamin D_3 (unlabeled) 50 µg/mL in ethanol CP 95%
ULM-9108-C	1,25-Dihydroxyvitamin D_3 (unlabeled) 100 µg/mL in ethanol CP 95%
ULM-9110	3-*epi*-25-Hydroxyvitamin D_2 (unlabeled)
ULM-9110-B	3-*epi*-25-Hydroxyvitamin D_2 (unlabeled) 50 µg/mL in ethanol
ULM-9110-C	3-*epi*-25-Hydroxyvitamin D_2 (unlabeled) 100 µg/mL in ethanol
DLM-9111	3-*epi*-25-Hydroxyvitamin D_3 (6,19,19-D_3, 98%)
DLM-9111-B	3-*epi*-25-Hydroxyvitamin D_3 (6,19,19-D_3, 98%) 50 µg/mL in ethanol
DLM-9111-C	3-*epi*-25-Hydroxyvitamin D_3 (6,19,19-D_3, 98%) 100 µg/mL in ethanol
ULM-9112	3-*epi*-25-Hydroxyvitamin D_3 (unlabeled)
ULM-9112-B	3-*epi*-25-Hydroxyvitamin D_3 (unlabeled) 50 µg/mL in ethanol
ULM-9112-C	3-*epi*-25-Hydroxyvitamin D_3 (unlabeled) 100 µg/mL in ethanol
CLM-9113	25-Hydroxyvitamin D_2 (25,26,27-$^{13}C_3$, 99%)
CLM-9113-B	25-Hydroxyvitamin D_2 (25,26,27-$^{13}C_3$, 99%) 50 µg/mL in ethanol
CLM-9113-C	25-Hydroxyvitamin D_2 (25,26,27-$^{13}C_3$, 99%) 100 µg/mL in ethanol
DLM-9114	25-Hydroxyvitamin D_2 (6,19,19-D_3, 97%)
DLM-9114-A	25-Hydroxyvitamin D_2 (6,19,19-D_3, 97%) 5 µg/mL in ethanol
DLM-9114-B	25-Hydroxyvitamin D_2 (6,19,19-D_3, 97%) 50 µg/mL in ethanol
DLM-9114-C	25-Hydroxyvitamin D_2 (6,19,19-D_3, 97%) 100 µg/mL in ethanol
ULM-9115	25-Hydroxyvitamin D_2 (unlabeled)
ULM-9115-A	25-Hydroxyvitamin D_2 (unlabeled) 5 µg/mL in ethanol
ULM-9115-B	25-Hydroxyvitamin D_2 (unlabeled) 50 µg/mL in ethanol
ULM-9115-C	25-Hydroxyvitamin D_2 (unlabeled) 100 µg/mL in ethanol
DLM-9116	25-Hydroxyvitamin D_3 (6,19,19-D_3, 97%)
DLM-9116-A	25-Hydroxyvitamin D_3 (6,19,19-D_3, 97%) 5 µg/mL in ethanol
DLM-9116-B	25-Hydroxyvitamin D_3 (6,19,19-D_3, 97%) 50 µg/mL in ethanol
DLM-9116-C	25-Hydroxyvitamin D_3 (6,19,19-D_3, 97%) 100 µg/mL in ethanol
DLM-7708	25-Hydroxyvitamin D_3 (26,26,26,27,27,27-D_6, 98%)
ULM-9117	25-Hydroxyvitamin D_3 (unlabeled)
ULM-9117-A	25-Hydroxyvitamin D_3 (unlabeled) 5 µg/mL in ethanol
ULM-9117-B	25-Hydroxyvitamin D_3 (unlabeled) 50 µg/mL in ethanol
ULM-9117-C	25-Hydroxyvitamin D_3 (unlabeled) 100 µg/mL in ethanol
DLM-9069	Pyridoxal•HCl (methyl-D_3, 98%)
CLM-320	Retinal (10-^{13}C, 99%)
CLM-325	Retinal (11-^{13}C, 99%)
CLM-326	Retinal (14-^{13}C, 99%)
CLM-327	Retinal (15-^{13}C, 99%)
DLM-7719	Retinal (D_6, 96%+)
CLM-331	Retinoic acid (10-^{13}C, 99%)
CLM-328	Retinoic acid (11-^{13}C, 98%)
CLM-329	Retinoic acid (14-^{13}C, 99%)
CLM-330	Retinoic acid (15-^{13}C, 99%)
CLM-4343	Retinoic acid (10,11,14,15-$^{13}C_4$, 99%)
DLM-7720	Retinoic acid (D_6, 96%+)
DLM-8113	Retinol (19,19,19,20,20,20-D_6, 97%+)
DLM-4902	Retinyl palmitate (+0.5 mg/mL BHT) (10,19,19,19-D_4, 96%)
CLM-8870	Vitamin A acetate (12,13,14,20-$^{13}C_4$, 99%)
CLM-4831	Vitamin A acetate (8,9,10,12,13,14,19,20-$^{13}C_8$, 99%)
CLM-7277	Vitamin A acetate (8,9,10,11,12,13,14,15,19,20-$^{13}C_{10}$, 99%)
DLM-2244	Vitamin A acetate 3-4% *cis* (10,19,19,19-D_4, 96%)
DLM-3828	Vitamin A acetate 3-4% *cis* (19,19,19,20,20,20-D_6, 96%)
DLM-4203	Vitamin A acetate 3-4% *cis* (10,14,19,19,19,20,20,20-D_8, 90%)
CLM-7667	Vitamin B_1 (Thiamine chloride) (4,5,4-methyl-$^{13}C_3$, 99%)
CNLM-8851	Vitamin B_2 (Riboflavin) ($^{13}C_4$, 99%; $^{15}N_2$, 98%) CP >97%
ULM-9123	Vitamin B_2 (Riboflavin) (unlabeled) CP 97%
CNLM-7694	Vitamin B_5 (Pantothenic acid, calcium salt monohydrate) (β-alanyl-$^{13}C_3$, 99%; ^{15}N, 98%)
ULM-9118	Vitamin B_6 (Pyridoxal•HCl) (unlabeled)
DLM-9119	Vitamin B_6 (Pyridoxamine•2HCl) (methyl-D_3, 98%)
ULM-9120	Vitamin B_6 (Pyridoxamine•2HCl) (unlabeled)
CLM-7563	Vitamin B_6 (Pyridoxine•HCl) (4,5-*bis* (hydroxymethyl)-$^{13}C_4$, 99%)
DLM-9121	Vitamin B_6 (Pyridoxine•HCl) (methyl-D_3, 98%) CP 96%
ULM-1992	Vitamin B_6 (Pyridoxine•HCl) (unlabeled)
ULM-9122	Vitamin B_6 (Pyridoxine•HCl) (unlabeled) CP 96%
CLM-7861	Vitamin B_9 (Folic acid) ($^{13}C_5$, 95%+) (contains ~10% H_2O)
DLM-8985	Vitamin D_2 (Ergocalciferol) (6,19,19-D_3, 97%)
ULM-9124	Vitamin D_2 (Ergocalciferol) (unlabeled)
ULM-9124-C	Vitamin D_2 (Ergocalciferol) (unlabeled) 100 µg/mL in ethanol
ULM-9124-D	Vitamin D_2 (Ergocalciferol) (unlabeled) 1000 µg/mL in ethanol
CLM-7850	Vitamin D_3 (Cholecalciferol) ($^{13}C_2$, 99%) CP 90%
DLM-8853-C	Vitamin D_3 (Cholecalciferol) (D_3, 97%) 100 µg/mL in ethanol CP 97%
DLM-8853-D	Vitamin D_3 (Cholecalciferol) (D_3, 97%) 1000 µg/mL in ethanol CP 97%

CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

(continued)

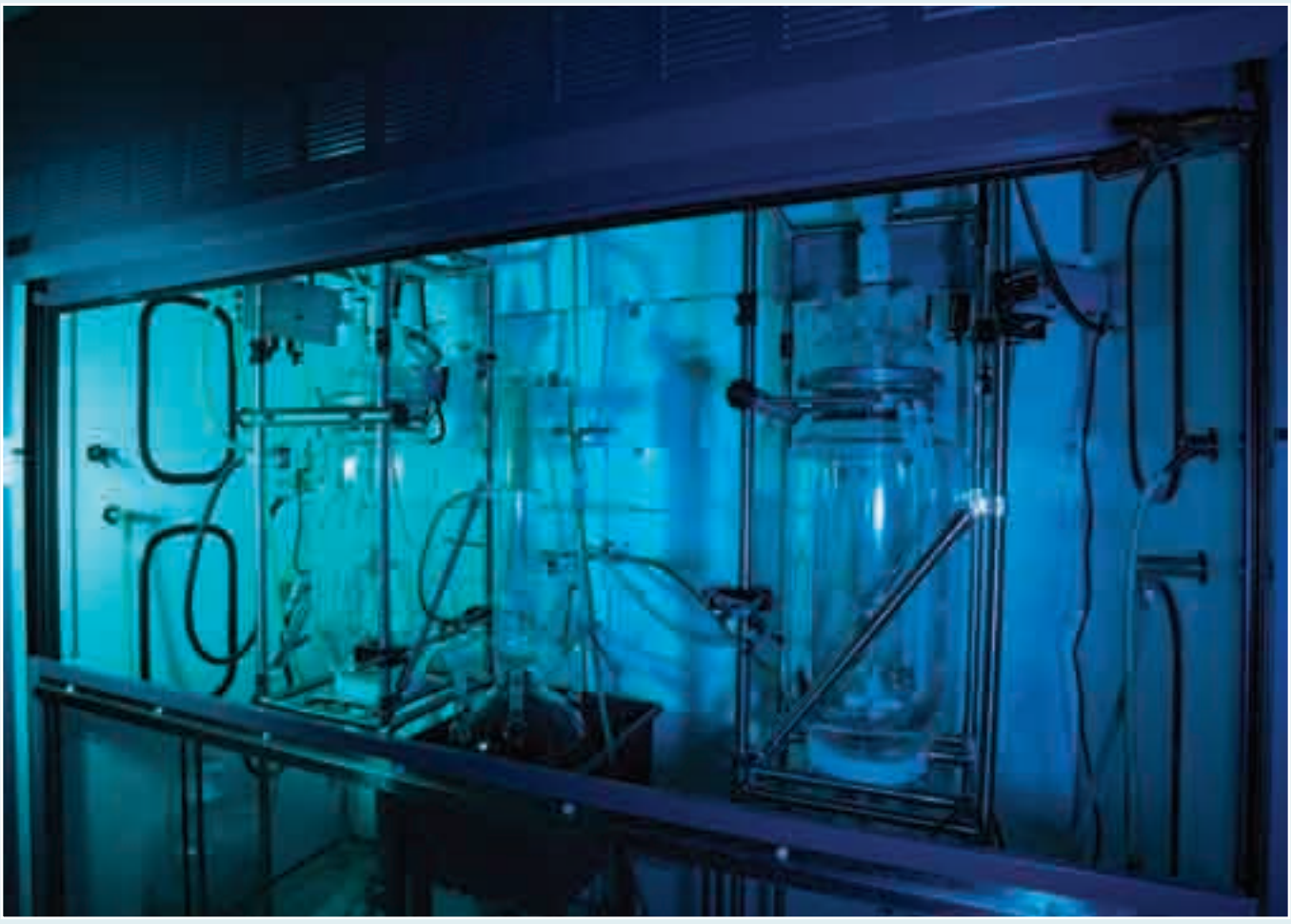
Vitamins

Catalog No.	Description
ULM-9125	Vitamin D_3 (Cholecalciferol) (unlabeled)
ULM-9125-C	Vitamin D_3 (Cholecalciferol) (unlabeled) 100 µg/mL in ethanol
ULM-9125-D	Vitamin D_3 (Cholecalciferol) (unlabeled) 1000 µg/mL in ethanol
DLM-9126	Vitamin E (α-Tocopherol) (5-methyl-D_3,7-methyl-D_3, 98%)
ULM-9127	Vitamin E (α-Tocopherol) (unlabeled)
DLM-8847	Vitamin E acetate (Tocopherol acetate) (acetyl-D_3, 98%)
DLM-9128	Vitamin H (Biotin) (2',2',3',3',4',4',5',5'-D_8, 99%)
ULM-9129	Vitamin H (Biotin) (unlabeled)
DLM-7702	Vitamin K_1 (Phylloquinone) (ring-D_4, 98%)
DLM-9130	Vitamin K_1 (Phylloquinone) (D_7, 99%) CP 97%
ULM-9131	Vitamin K_1 (Phylloquinone) (unlabeled) CP 97%
DLM-9132	Vitamin K_3 (Menadione) (D_8, 98%) CP 97%
ULM-9133	Vitamin K_3 (Menadione) (unlabeled) CP 97%

Water

Catalog No.	Description
DLM-4-70	Deuterium oxide (D, 70%)
DLM-4-99	Deuterium oxide (D, 99%)
DLM-4-99.8	Deuterium oxide (D, 99.8%)
DLM-4	Deuterium oxide (D, 99.9%)
DLM-6	Deuterium oxide "100%" (D, 99.96%)
DLM-11	Deuterium oxide (D, 99.9%) low paramagnetic
DOLM-242	Water (D_2, 98%; ^{18}O, 97%)
OLM-240-10	Water (^{18}O, 10%)
OLM-240-50	Water (^{18}O, 50-60%)
OLM-240-97	Water (^{18}O, 97%)
OLM-240-99	Water (^{18}O, 99%)
OLM-782-10	Water (^{17}O, 10%)
OLM-782-20	Water (^{17}O, 20%)
OLM-782-40	Water (^{17}O, 35-40%)
OLM-782-70	Water (^{17}O, 70%)
OLM-782-85	Water (^{17}O, 85%)
OLM-782-90	Water (^{17}O, 90%)

Custom double-labeled water (^{18}O; D) also available.



CIL also offers microbiological and pyrogen-tested products. Please see pages 92-93 for a complete listing.

CIL would like to acknowledge and thank the many talented researchers who graciously contributed their time and expertise to assist in the production of this catalog.

John M. Asara, PhD
Gary Bellinger
Ian Blair, PhD
Professor Yves Boirie
Joel B. Braunstein, MD, MBA
Susanne B. Breitkopf, PhD
Prof. John W. Brock, PhD
Kristy J. Brown, PhD
Michael Burgess
Dr. Steven A. Carr
Jose Castro-Perez, PhD
Donald H. Chace, PhD, MSFS, FACB
Eugene Ciccimaro, PhD
Amy Claydon, PhD
Michele Cleary, PhD
Dr. Joshua J. Coon
Tom Crabtree
Simon J. Davies, PhD
Mary K. Doherty, PhD
Dr. Catherine Fenselau
Chang-Gyu Hahn, MD, PhD
Yetrib Hathout, PhD
Marc Hellerstein, MD, PhD
Kithsiri Herath, PhD
Douglas G. Johns, PhD
Dr. David C. Kasper
Dr. Karsten Kuhn
Joel Louette, MS
Costas A. Lyssiotis, PhD
Michael J. MacCoss, PhD
Matthew L. MacDonald, PhD
Ablatt Mahsut, PhD
Juan C. Marini, DVM, PhD
Samuel Massoni, CEO
Dwight E. Matthews, PhD
Daniel B. McClatchy, PhD
David G. McLaren, PhD
Zhaojing Meng, PhD
Dr. Marc Moniatte
Paul L. Miller
Ron Orlando, PhD
Akhilesh Pandey, PhD
Sung Kyo (Robin) Park, PhD
Donald G. Patterson, Jr., PhD
Tero Pihlajamaa, PhD
Stephen F. Previs, PhD
Dr. Iain Pritchard
Jenson Qi, PhD
Joshua D. Rabinowitz, PhD
Dr. Fred E. Regnier
Serge Roche, PhD
Thomas P. Roddy, PhD
Jeffrey Savas, PhD
Vinit Shah, MS
Brad Silverbush
Gabriel M. Simon, PhD
Audrey Sirvent, PhD
Matthew Steinhauser, MD
Steven J. Stout, PhD
Serge Urbach, PhD
Timothy D. Veenstra, PhD
Dan Vickers
Tim West, PhD
Sheng-Ping Wang
Phillip D. Whitfield, PhD
Robert R. Wolfe, PhD
John R. Yates, III, PhD
Iain S. Young, PhD
Min Yuan, PhD
Ming Zhou, PhD

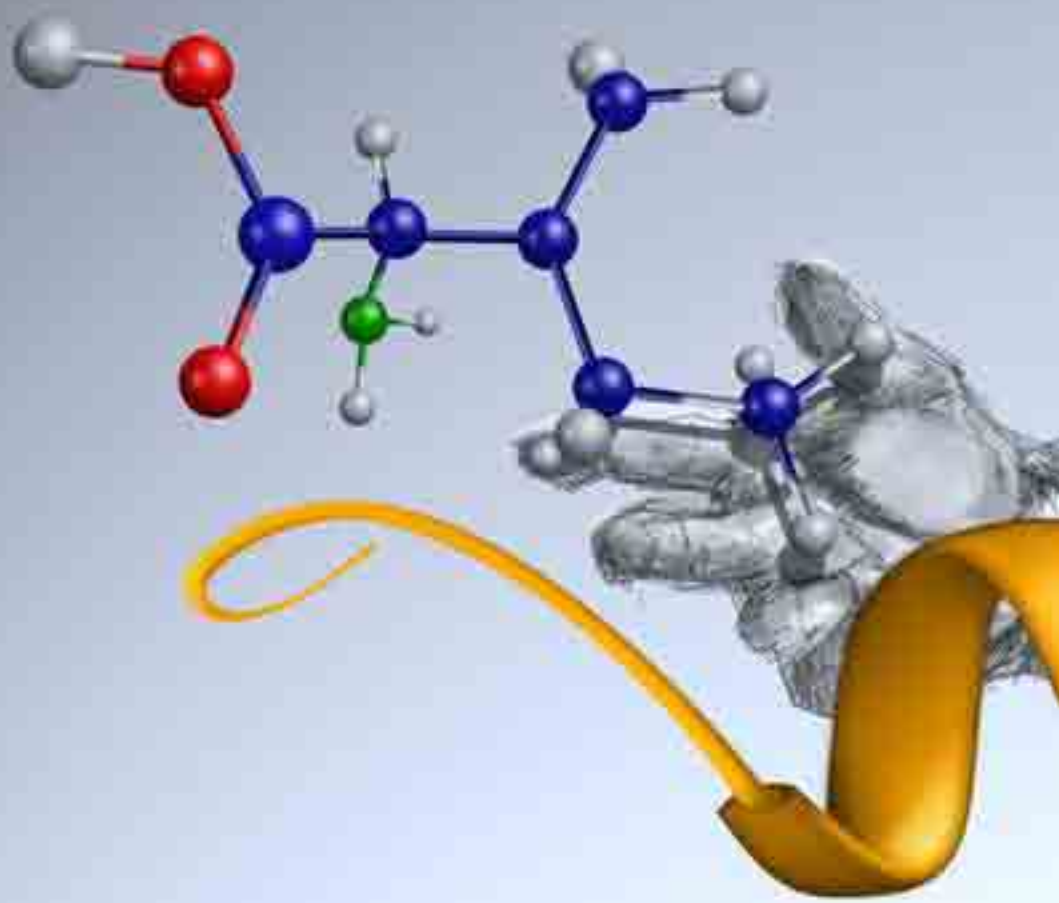
BUCHEM BV

www.isotope.nl

Orders BENELUX

Buchem B.V.
Minden 60/62
7327 AW Apeldoorn
The Netherlands

Tel +31 (0)55 543 00 40
Fax +31 (0)55 543 00 50
Email info@buchem.com
Web www.buchem.com
www.isotope.nl



Printed with soy-based inks
5.13

Cambridge Isotope Laboratories, Inc.

50 Frontage Road, Andover, MA 01810-5413 USA
Tel: 800.322.1174 (North America) Tel: 978.749.8000
Fax: 978.749.2768
www.isotope.com