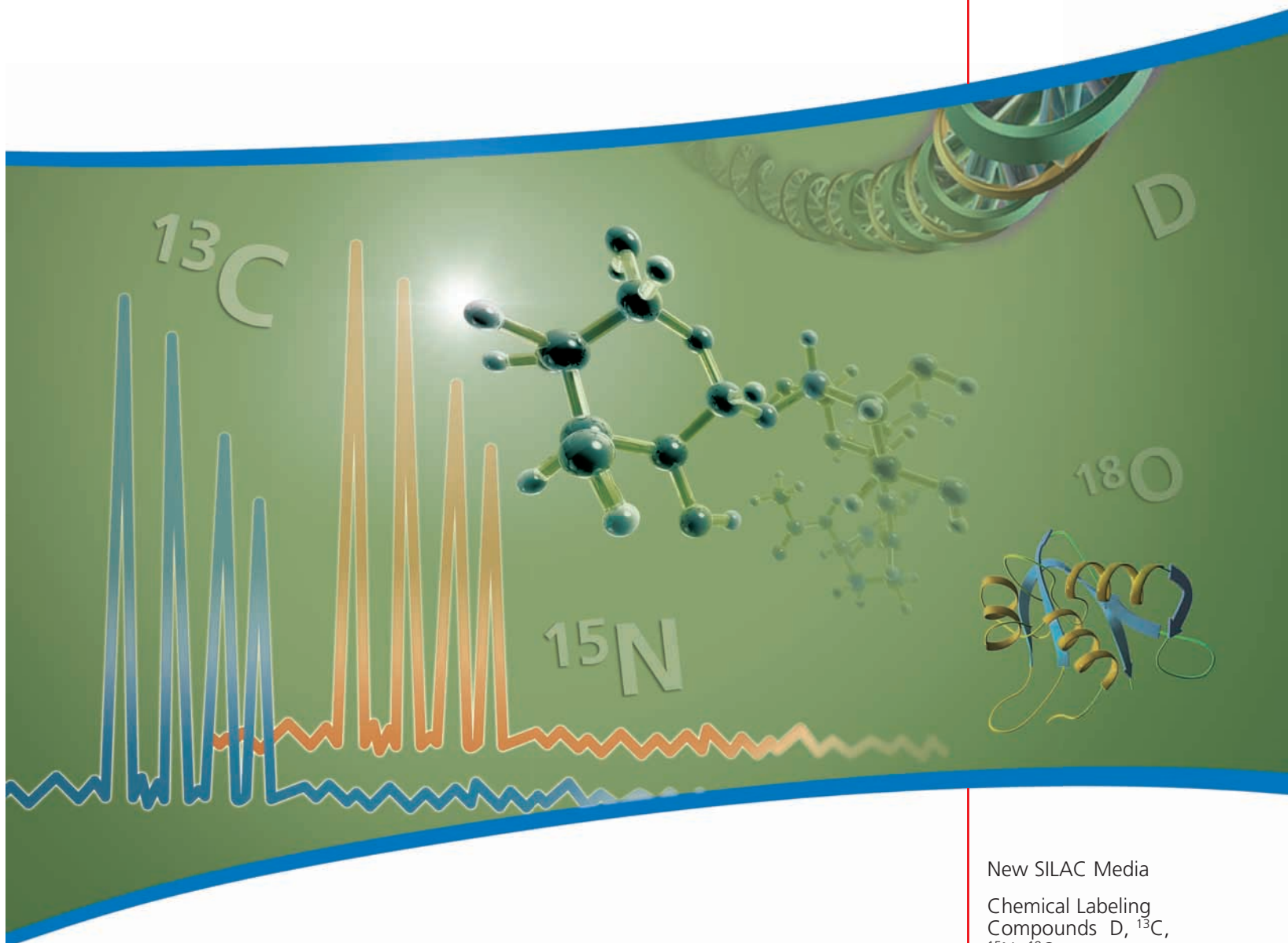


ISOTECH[®] Stable Isotopes

Expanding the Boundaries of
Quantitative Proteomics



New SILAC Media

Chemical Labeling
Compounds D, ¹³C,
¹⁵N, ¹⁸O

Free and Protected
Amino Acids

Enzymatic Labeling with
Water- ¹⁸O

The Utility of Stable Isotopes in Contemporary Proteomics

While genomic studies provide insight into the roles of DNA and gene expression in biological systems, ultimately it is the change in the concentration, localization, or identity of the effectors of biological function and proteins, that must be analyzed. To this end, proteomics, which is defined as the examination of the global protein content of a biological system under specific conditions, has evolved as an invaluable tool for characterizing the complexity of living organisms. Proteomics-derived data have proven to be of interest in the identification and development of novel biomarkers for diagnostic and therapeutic applications. It has been of particular importance with regard to the characterization of differential protein levels in normal and pathophysiological states for various human illnesses including cancer⁽¹⁾ and cardiovascular disease⁽²⁻³⁾. Proteomics holds tremendous potential to increase the molecular understanding of disease pathogenesis as evidenced by recent advances toward the quantification of alterations in protein abundance using mass spectrometry (MS).

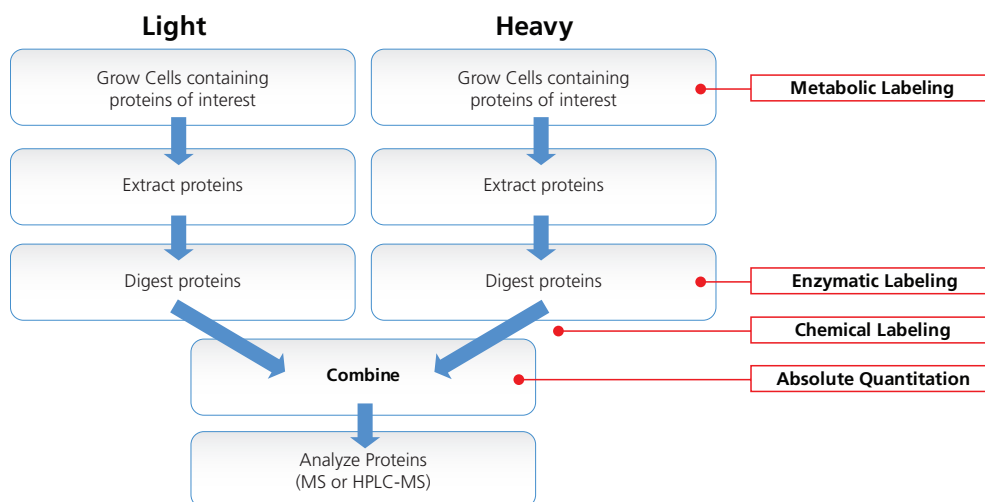
The usage of stable isotope labeling and MS in proteomics facilitates the quantitation of changes in protein levels in biological systems. As such, quantitative proteomics involves the determination of absolute differences in global protein expression of cells, often as a consequence of endogenous or exogenous stimuli. The combined application of stable isotope labeling and MS in proteomics has enabled researchers to make quantitative comparisons in protein levels between multiple biological samples. Incorporation of "heavy" stable isotopes such as ¹³C, D, and ¹⁵N into proteins for mass spectrometric analysis is accomplished by the attachment of site-specific tags⁽⁴⁾, metabolic labeling⁽⁵⁾, and enzymatic reactions⁽⁶⁻⁸⁾. For quantitative methodologies, two separate samples – one produced with "heavy" isotopes used as internal controls and the other with "light" or the natural abundance isotopes – are examined. The samples are combined prior to mass spectrometric analysis and doublet peaks observed in the mass spectrum originating from identical "light", and "heavy" peptide fragments are compared. The ratio of the "light" and "heavy" isotopic peak intensities for a particular peptide provides relative measurement of protein abundance in a given spectrum. This approach permits the simultaneous evaluation of numerous proteins from defined biological states. Advances in global metabolic labeling methodologies combined with improved instrumentation greatly expand the scope and potential of quantitative proteomics.

We remain committed to advancing scientific study by providing the MS community with high-quality, isotopically enriched products for use in quantitative proteomics studies. We offer a full complement of products to enzymatically, chemically, or metabolically stable isotope-label your proteins. For unique applications or custom synthesis needs, our team of scientific experts is always available to assist in the design of your stable isotope labeled biomolecule of interest.

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Overview of Stable Isotope Usage in Protein Labeling



Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) A Primer

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What is SILAC?

Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) was developed to monitor the relative abundance of proteins by mass spectrometry (1). This method works on the premise that cell treatment with light (^{12}C and/or ^{14}N) and heavy isotope (^{13}C and/or ^{15}N) labeled amino acids gives rise to two almost identical proteomes, which – under the same cell culture conditions – differ only in their masses. Deuterium is used to a lesser extent as deuterated compounds are often resolved from the non-deuterated compounds by reversed-phase liquid chromatography. This adversely affects quantitation when performing LC/MS experiments. Due to this substitution a mass increment is observed in the mass spectra for each peptide comprising at least one of the heavy isotope labeled amino acids (e.g. 10 Da for $^{13}\text{C}_6$, $^{15}\text{N}_4$ -Arg). The advantages of this method over alternative derivatization-based labeling techniques (such as Isotope-Coded Affinity Tag, ICATTM) is that the incorporation of light and heavy isotopes takes place in the proteome of living cells before a given biological experiment (e.g. stimulating cells with a cytokine). Thus, it is possible to combine the cells directly after harvesting them for subsequent purification steps and analysis. This ensures maximum reproducibility and minimum sample variation with regard to the protein level.

How does SILAC work?

The basis of SILAC is the incorporation of a stable isotope containing amino acid into the whole proteome. A typical SILAC experiment is designed in a differential manner, thus allowing the comparison of different cellular states such as stimulated vs. non-stimulated or at various time points under identical biological conditions. As the two isotopically labeled amino acids are essentially chemically identical, their incorporation does not interfere with normal cell growth, while leading to proteins/peptides that are distinguishable by mass and thus are ideal for mass spectrometric analysis. By choosing the right heavy amino acids it is possible to multiplex up to three different conditions (e.g. Arg; $^{13}\text{C}_6$ -Arg; $^{13}\text{C}_6$, $^{15}\text{N}_4$ -Arg). The SILAC samples are then subjected to enzymatic digestion and LC/MS analysis (in a typical bottom up proteomics approach). The protein quantification is therefore carried out on the peptide level by comparing the peak height or area of the corresponding doublets i.e. peptides which have the same amino acid composition and sequence but different masses. The complete incorporation of the heavy isotope is achieved even for proteins with a low turn-over after five doublings. This is sufficient to exclude any partially labeled artifacts for MS-based quantification (1).

In order to obtain sufficient incorporation of the heavy isotope, a typical SILAC experiment is divided into two stages. In the first stage the cells are fed with the stable isotope labeled amino acids. To ensure the exclusive incorporation of the heavy isotopic labeled amino acid the following points have to be addressed:

- Ideally, the substituted amino acid should be essential to guarantee that the cell relies on an external source of this amino acid. The most frequently used essential amino acids are leucine (1) lysine and methionine. In addition to these essential amino acids, arginine has often and successfully been applied to SILAC experiments despite the fact that it is a non-essential amino acid (2); the availability of exogenous arginine is probably responsible for a down-regulation of arginine biosynthesis. The combined use of e.g. lysine and arginine in conjunction with tryptic digestion lead to a complete labeling of all tryptic peptides (except for the C-terminal peptide). The comprehensive coverage is obtained through the specificity of trypsin to cleave C-terminal to lysine and arginine.
- Cells have to be grown in the presence of dialyzed serum to minimize the contamination of non heavy isotope labeled amino acids.
- The use of heavy arginine was reported to lead to partial labeling of proline through metabolic conversion. This conversion results in multiple satellite peaks for all proline-containing tryptic peptides in the heavy state, which in turn affects the accuracy of quantitation. Recently, Krijgsveld *et al.* reported an experimental strategy to correct for this artifact. By using [$^{15}\text{N}_4$]-arginine in combination with light lysine in the light condition and [$^{13}\text{C}_6$, $^{15}\text{N}_4$]-arginine in combination with [$^{13}\text{C}_6$, $^{15}\text{N}_2$]-lysine in the heavy condition, heavy proline will be formed at the same rate under both conditions (that is, [$^{15}\text{N}_4$]-proline and [$^{13}\text{C}_6$, $^{15}\text{N}_1$]-proline, respectively), thus providing an internal correction for arginine conversion(3).

Advantages of SILAC

- No *in vitro* labeling steps are necessary.
- Both amino acids share the same physico-chemical properties
- No differences in the labeling efficiency are expected
- Compared with metabolic labeling using heavy amino acids is sequence specific and results in a constant mass shift
- The introduction of labeled amino acids leads to an excellent prediction of mass-labeled peptides
- The detection of several labeled peptides derived from the same protein enables better statistics to quantify the protein level and therefore better confidence in the measurements (1)

Shortcomings of SILAC

- Division of the ion current in LC/MS experiments in two signals
 - SILAC is limited to cell culture and labeling of whole organisms (such as *C. elegans* and *D. melanogaster*)(14)
 - Increase of the sample complexity due to the duplets
 - The multiplexing is limited to 3 different conditions
 - The dialyzed FBS might have an influence on the cell fitness.
-

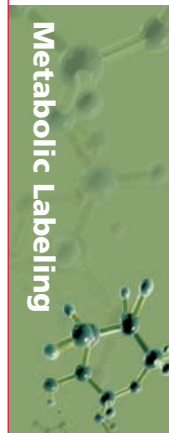
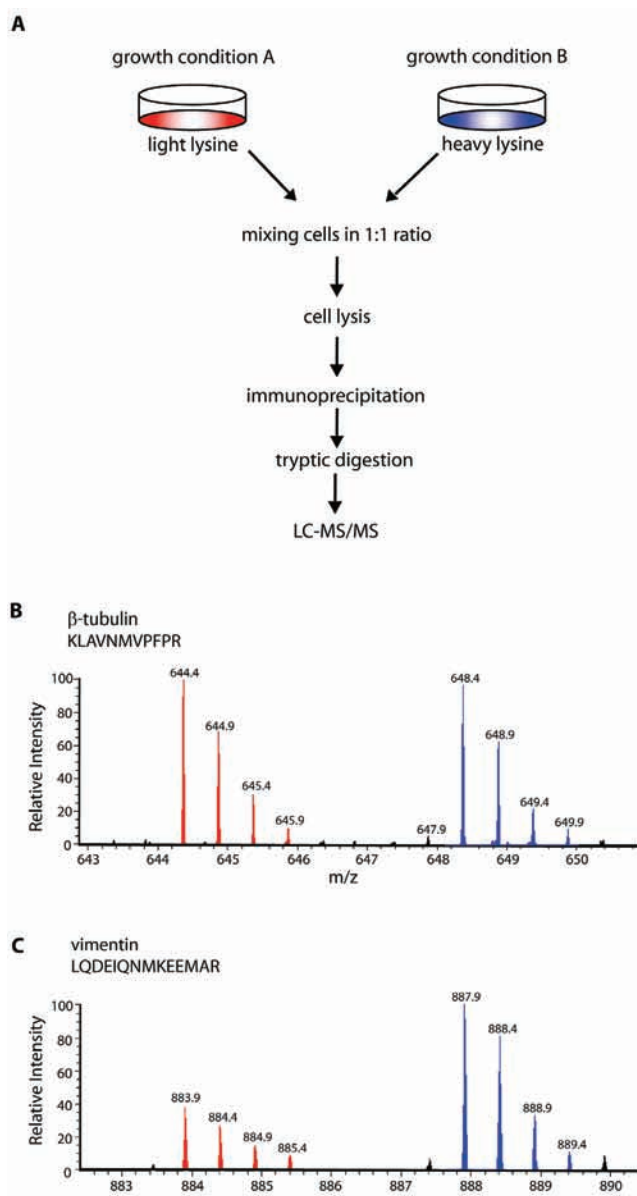


Figure A shows a SILAC workflow, where cells from two different cell stages are grown in light lysine and heavy lysine ($^{13}\text{C}_6, ^{15}\text{N}_2$ -lysine) containing media. The cell lysates generated under the two different conditions were combined in a 1:1 ratio prior to co-immunoprecipitating the interactors of Aurora-B kinase.

Mass spectrometry data from two different proteins are shown in Figure B and C. Tubulin did not show a significant difference between the light and heavy forms (ratio ~ 1.0) whereas vimentin is clearly more abundant in heavy labeled, stage B cells (ratio ~ 2.5) indicating that the association of vimentin to this protein complex is cell cycle dependent.



Examples for SILAC Applications

SILAC has been widely used to compare proteomes of different cell populations such as cells with and without cytokine stimulation, RNAi knock-down cells vs. wild type or disease vs. normal cells (for details see review Mann, 2006 (4)).

Everley *et al.* used the SILAC technology to compare the protein composition of two prostate cancer cell lines, which differ in their metastatic potential. This differential set up aimed to correlate the protein changes with the different metastasis ability of these cell lines (5).

Another example of a differential SILAC set up was used to determine the cytosolic interaction partners of all four ErbB receptor family members (6). Due to the usage of stable isotope labeled amino acids it was possible to distinguish between false positive and bait specific interactions both of which are easily detected by mass spectrometry. Any protein that shows a ratio of 1 between controls (i.e. unstimulated or only tag expressing cells) and the real sample (stimulated or bait tagged expressing cells) can be assigned as background protein. Whereas proteins that specifically interact with the bait will show a ratio significantly different from 1 (7, 8). Recently Wang *et al.* pointed out that specific but dynamic interactors may not be distinguished from the background proteins. Dynamic interactors result in an equilibrium between two isotopic labeled forms bound to the bait due to the fast on/off rates so the ratio would be close to 1. Thus, protein purifications both before and after mixing the cell lysates are advisable (9).

In addition to the determination of protein levels, SILAC approaches are well suited for monitoring changes in post-translational modifications. Examples for these applications include the measurement of changes in protein phosphorylation and methylation.

The utility of SILAC approach in the study of phosphorylation dynamics was demonstrated by Olsen *et al.*, who examined phosphorylation dynamics in response to EGF (epidermal growth factor) by using three different arginine isotopes to label cells. This approach facilitated the comparison of three different time points upon EGF stimulation. They reported the temporal profiles of more than 6500 phosphorylation sites upon growth factor stimulation (10).

Another example for the use of SILAC for the quantification of protein modifications was presented by Ong *et al.* They reported a "heavy methyl SILAC" strategy where methylation sites were directly labeled by growing cells under light and heavy methionine conditions. This approach provided more confidence in detection and quantification of protein methylation since the methylated peptides were present in pairs separated by the mass difference of the labeled methyl groups. Using this strategy, it was possible to describe 59 unique methylation sites on 33 different proteins in HeLa cells (11).

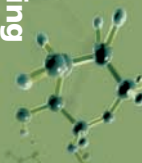
The use of SILAC for more comprehensive quantitation of several protein modifications was recently demonstrated by Bonenfant *et al.* and Vermeulen *et al.* Both groups used SILAC for the study of various histone modifications. While Bonenfant *et al.* used the SILAC approach for a comprehensive analysis of the dynamics of histone modifications (i.e. acetylation, methylation and phosphorylation) changes through cell cycle (12), Vermeulen *et al.* took a SILAC based histone peptide pull-down approach to screen specific interactors of histone H3 trimethylated on Lys-4 (H3K4me3). They showed that basal transcription factor TFIID specifically binds to H3K4me3. Using triple SILAC pull-down assays they further showed that H3dimethylation on Arg-2 inhibits TFIID binding to H3K4me3, whereas acetylation facilitates this interaction (13).

Summary

In summary, SILAC has proved to be a powerful method to quantify the relative differential changes in protein complexes. Due to the fact that the isotopic labels are introduced very early during normal cell growth, SILAC has the great advantage of carrying all the steps from purification to data analysis together with the proper internal control.

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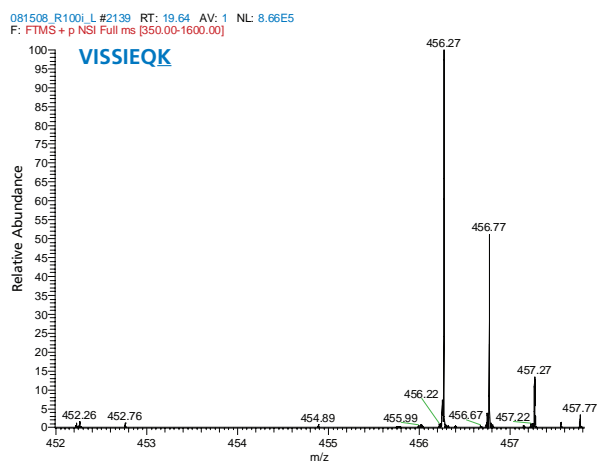
NEW SILAC Depleted Media

We now offer our exclusively formulated SILAC medias, which are deficient in arginine, leucine, and lysine. The Dulbecco's Modified Eagle's Medium contains low glucose (1000mg/L L-glucose), L-glutamine, and sodium bicarbonate without arginine, leucine, lysine, sodium pyruvate, and phenol red for SILAC labeling experiments. It is sterile filtered, cell culture tested, and offers the ultimate in stable isotope labeling flexibility. Also intended for SILAC applications, the specially formulated RPMI-1640 medium contains L-glutamine and sodium bicarbonate, but lacks arginine, leucine, lysine, and phenol red. This product is sterile filtered and cell culture tested. It permits greater adaptability in metabolic labeling applications. In addition, we have a complete selection of natural abundance and stable isotope labeled amino acids for metabolic labeling.

Incorporation Efficiency for DME and RPMI SILAC Medias

Cells (HeLa or MCR-5) were grown for approximately six doublings with routine media exchange using 100mg/L each of heavy arginine and heavy lysine. Cell pellets were lysed and subjected to SDS-PAGE analysis. Protein bands were extracted and digested with trypsin. Validation experiments were performed using a LTQ-FT mass spectrometer to confirm isotopic enrichment. An incorporation efficiency of 98 atom % was achieved using heavy L-lysine- $^{13}\text{C}_6$, $^{15}\text{N}_2$ (Figure A) and heavy L-arginine- $^{13}\text{C}_6$, $^{15}\text{N}_4$ (Figure B).

Figure A



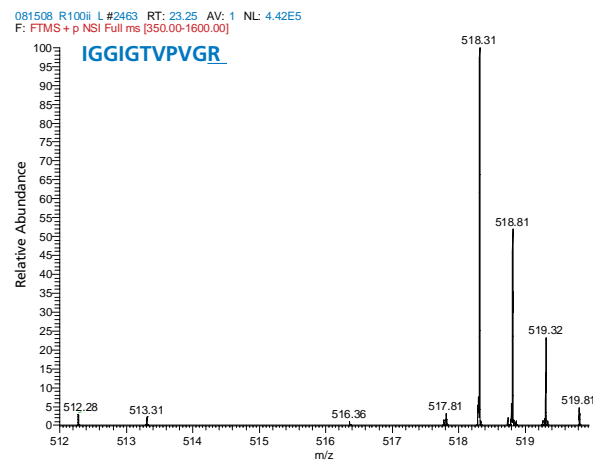
Advantages of SILAC Media include:

- >98 atom % isotopic enrichment using 100 mg/L of heavy amino acids
- Media depleted of arginine, lysine, and leucine offers greatest labeling flexibility and sequence coverage
- Suitable for a variety of SILAC applications

Depleted Media

| Cat. No. | Name |
|----------|---|
| D9443 | Dulbecco's Modified Eagle's Medium - low glucose, With 1000mg/L L-glucose, L-glutamine, and sodium bicarbonate. Without arginine, leucine, lysine, sodium pyruvate, and phenol red, liquid, sterile-filtered, cell culture tested |
| R1780 | RPMI-1640 Medium With L-glutamine and sodium bicarbonate. Without arginine, leucine, lysine, and phenol red, liquid, sterile-filtered, cell culture tested |

Figure B



Primary "Stable Isotope Labeled" Amino Acids

| Cat. No. | Name | Isotopic Purity |
|----------|---|--|
| 643440 | L-Arginine- $^{13}\text{C}_6$ hydrochloride | 98 atom % ^{13}C |
| 608033 | L-Arginine- $^{13}\text{C}_6$, $^{15}\text{N}_4$ hydrochloride | 98 atom % ^{15}N 98 atom % ^{13}C |
| 600113 | L-Arginine- $^{15}\text{N}_4$ hydrochloride | 98 atom % ^{15}N |
| 608092 | L-Isoleucine- $^{13}\text{C}_6$, ^{15}N | 98 atom % ^{13}C 98 atom % ^{15}N |
| 486825 | L-Leucine-5,5,5- d_3 | 99 atom % D |
| 605239 | L-Leucine- $^{13}\text{C}_6$ | 98 atom % ^{13}C |
| 608068 | L-Leucine- $^{13}\text{C}_6$, ^{15}N | 98 atom % ^{13}C 98 atom % ^{15}N |
| 616192 | L-Lysine-4,4,5,5- d_4 hydrochloride | 98 atom % D |
| 643459 | L-Lysine- $^{13}\text{C}_6$ hydrochloride | 98 atom % ^{13}C |
| 608041 | L-Lysine- $^{13}\text{C}_6$, $^{15}\text{N}_2$ hydrochloride | 98 atom % ^{13}C 98 atom % ^{15}N |
| 608106 | L-Methionine- $^{13}\text{C}_5$, ^{15}N | 98 atom % ^{13}C 98 atom % ^{15}N |

| Cat. No. | Name | Isotopic Purity |
|----------|---|--|
| 608149 | L-Methionine-1- ^{13}C , d_3 (carboxy- ^{13}C , methyl- d_3) | 99 atom % ^{13}C 99 atom % D |
| 299154 | L-Methionine- ^{13}C , d_3 (methyl- ^{13}C , d_3) | 99 atom % ^{13}C 99 atom % D |
| 300616 | L-Methionine- d_3 (methyl- d_3) | 98 atom % D |
| 608017 | L-Phenylalanine- $^{13}\text{C}_9$, ^{15}N | 98 atom % ^{13}C 98 atom % ^{15}N |
| 607770 | L-Threonine- $^{13}\text{C}_4$, ^{15}N | 98 atom % ^{13}C 98 atom % ^{15}N |
| 489794 | L-Tyrosine- $^{13}\text{C}_9$ (phenyl- $^{13}\text{C}_6$) | 99 atom % ^{13}C |
| 492868 | L-Tyrosine- $^{13}\text{C}_9$ | 98 atom % ^{13}C |
| 607991 | L-Tyrosine- $^{13}\text{C}_9$, ^{15}N | 98 atom % ^{13}C 98 atom % ^{15}N |
| 600148 | L-Valine- $^{13}\text{C}_5$, ^{15}N | 98 atom % ^{13}C 98 atom % ^{15}N |

Primary "Natural Abundance" Amino Acids

| Cat. No. | Name |
|----------|---|
| A6969 | L-Arginine monohydrochloride meets EP, JP, USP testing specifications, cell culture tested, not synthetic |
| I7403 | L-Isoleucine meets EP, JP, USP testing specifications, cell culture tested, from non-animal source |
| L8912 | L-Leucine meets EP, JP, USP testing specifications, cell culture tested, from non-animal source |
| L8662 | L-Lysine monohydrochloride meets EP, JP, USP testing specifications, cell culture tested, from non-animal source |

| Cat. No. | Name |
|----------|---|
| M5308 | L-Methionine meets EP, JP, USP testing specifications, cell culture tested, from non-animal source |
| T8441 | L-Threonine cell culture tested, meets EP, JP, USP testing specifications, from non-animal source |
| T8566 | L-Tyrosine cell culture tested, meets EP, USP testing specifications, from non-animal source |
| V0513 | L-Valine meets EP, JP, USP testing specifications, cell culture tested, from non-animal source |

Additional Metabolic Labeling Products

| Cat. No. | Name | Isotopic Purity |
|----------|---|--|
| 299251 | Ammonium- ¹⁵ N chloride | 98 atom % ¹⁵ N |
| 366501 | Ammonium- ¹⁵ N, d ₄ chloride | 98 atom % ¹⁵ N 99 atom % D |
| 488011 | Ammonium- ¹⁵ N hydroxide solution ~ 3 N in H ₂ O, | 98 atom % ¹⁵ N |
| 299286 | Ammonium- ¹⁵ N ₂ sulfate | 98 atom % ¹⁵ N |
| 593990 | Ammonium- ¹⁵ N ₂ , d ₄ sulfate | 99 atom % ¹⁵ N 98 atom % D |
| 617385 | Deuterium oxide | 99.8 atom % D |
| 552003 | D-Glucose-C-d ₇ | 97 atom % D |
| 389374 | D-Glucose- ¹³ C ₆ | 99 atom % ¹³ C |
| 552151 | D-Glucose- ¹³ C ₆ , C-d ₇ | 99 atom % ¹³ C 97 atom % D |

| Cat. No. | Name | Isotopic Purity |
|----------|--|--|
| 606863 | ISOGRO® ¹³ C Powder -Growth Medium | 99 atom % ¹³ C |
| 606839 | ISOGRO ¹³ C, ¹⁵ N Powder -Growth Medium | 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 608297 | ISOGRO ¹³ C, ¹⁵ N, D Powder -Growth Medium | 99 atom % ¹³ C 98 atom % ¹⁵ N 97-99 atom % D |
| 606871 | ISOGRO ¹⁵ N Powder -Growth Medium | 98 atom % ¹⁵ N |
| 608300 | ISOGRO ¹⁵ N,D Powder -Growth Medium | 98 atom % ¹⁵ N 97 atom % D |
| 608750 | Potassium nitrate- ¹⁴ N | 99.95 atom % ¹⁴ N |
| 335134 | Potassium nitrate- ¹⁵ N | 98 atom % ¹⁵ N |
| 372382 | Sodium bicarbonate- ¹³ C | 98 atom % ¹³ C |

Additional Products of Interest

| Cat. No. | Name |
|----------|---|
| A6283 | Acetic acid <i>ReagentPlus</i> ®, ≥99% |
| B2025 | Brilliant Blue G - Colloidal Concentrate |
| F0392 | Fetal Bovine Serum Dialyzed by ultrafiltration against 0.15 M NaCl, USA Yes, sterile-filtered, cell culture tested |
| G7513 | L-Glutamine solution 200mM, solution, sterile-filtered, cell culture tested |
| I6125 | Iodoacetamide crystalline |
| M3641 | Methanol spectrophotometric grade, ≥99% |

| Cat. No. | Name |
|----------|---|
| P4333 | Penicillin-Streptomycin 100 ×, liquid, stabilized, sterile-filtered, cell culture tested |
| S8157 | Silver nitrate SigmaUltra, >99% (titration) |
| S2127 | Sodium carbonate <i>ReagentPlus</i> , ≥99% |
| L5750 | Sodium dodecyl sulfate ~95% based on total alkyl sulfate content basis |
| T3924 | Trypsin-EDTA solution 1 ×, 0.5 g porcine trypsin and 0.2 g EDTA • 4Na per liter of Hanks' Balanced Salt Solution with phenol red., sterile-filtered, cell culture tested |

Chemical Labeling

Stable isotope coded labels enable researchers to perform mass spectrometric- based proteomics studies in the absence of metabolic labeling. Labeling occurs by site-specific incorporation of stable isotope labeled tags at cysteine residues or the general labeling of amines and carboxyl groups in protein samples. These techniques are particularly useful for applications where metabolic labeling is impractical or undesirable such as clinical samples. Many methods for the chemical derivatization of proteins exist ⁽¹⁻³⁾ offering researchers the ability to optimize stable isotope label incorporation based on target proteins. In addition to stable isotope labeled tags, the development of innovative matrixes and improvements in instrumentation greatly impact stable isotope usage in quantitative proteomics ⁽⁴⁻⁵⁾. ISOTEC has the high-quality isotopically enriched products needed for mass-spectrometry based quantitative proteomics. Consult with our R&D scientists to develop novel stable isotope labeled compounds for use in comparative proteomic applications.

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Chemical Labeling Products

| Cat. No. | Name | Isotopic Purity |
|----------|--|--|
| 176567 | Acetaldehyde-d ₄ | 98 atom % D |
| 531227 | Acetaldehyde- ¹³ C ₂ | 99 atom % ¹³ C |
| 607452 | Acetic anhydride-1,1'- ¹³ C ₂ ,d ₆ | 99 atom % ¹³ C 99 atom % D |
| 487821 | Acetic anhydride- ¹³ C ₄ | 99 atom % ¹³ C |
| 607428 | Acetic anhydride- ¹³ C ₄ ,d ₆ | 99 atom % ¹³ C 97 atom % D |
| 633259 | N-Acetoxy-d ₃ -succinimide | 98 atom % D |
| 607517 | Acetyl chloride-1- ¹³ C,d ₃ | 99 atom % ¹³ C 98 atom % D |
| 636568 | Acrylamide-2,3,3-d ₃ | 98 atom % D |
| 577820 | Acrylamide-1- ¹³ C | 99 atom % ¹³ C |
| 586617 | Acrylamide- ¹³ C ₃ | 99 atom % ¹³ C |
| 485691 | Benzoic- ¹³ C ₆ acid (ring- ¹³ C ₆) | 99 atom % ¹³ C |
| 366048 | Benzoyl chloride-d ₅ | 99 atom % D |
| 279323 | Benzoyl chloride-α- ¹³ C | 99 atom % ¹³ C |
| 283835 | Bromoacetic acid- ¹³ C ₂ | 99 atom % ¹³ C |
| 485209 | 2-Bromoethanol-1,1,2,2-d ₄ | 98 atom % D |
| 600024 | 2-Bromoethanol- ¹³ C ₂ ,1,1,2,2-d ₄ | 99 atom % ¹³ C 98 atom % D |
| 614491 | 3-Bromo-1-propan-d ₆ -ol | 98 atom % D |
| 604089 | Chloroacetyl chloride- ¹³ C ₂ | 99 atom % ¹³ C |
| 491535 | 4-Chlorobenzaldehyde-2,3,5,6-d ₄ | 98 atom % D |
| 164526 | Dimethyl sulfate-d ₆ | 99 atom % D |
| 485500 | Dimethyl sulfate- ¹³ C ₂ | 99 atom % ¹³ C |
| 590096 | Dimethyl sulfate- ¹³ C ₂ ,d ₆ | 98 atom % D 99 atom % ¹³ C |

| Cat. No. | Name | Isotopic Purity |
|----------|--|--|
| 457833 | Ethylene-d ₄ oxide | 98 atom % D |
| 492620 | Formaldehyde-d ₂ solution ~20 wt. % in D ₂ O | 98 atom % D |
| 489417 | Formaldehyde- ¹³ C solution 20 wt. % in H ₂ O | 99 atom % ¹³ C |
| 596388 | Formaldehyde- ¹³ C, d ₂ solution 20 wt. % in D ₂ O | 99 atom % ¹³ C 98 atom % D |
| 607312 | Guanidine- ¹³ C, ¹⁵ N ₃ hydrochloride | 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 592668 | Iodoacetamide- ¹⁵ N | 98 atom % ¹⁵ N |
| 595489 | Iodoacetic acid- ¹³ C ₂ | 99 atom % ¹³ C |
| 294756 | Iodomethane- ¹³ C,d ₃ | 99.5 atom % D 99 atom % ¹³ C |
| 493171 | O-Methylisourea- ¹³ C hydrochloride | 99 atom % ¹³ C |
| 608467 | O-Methylisourea- ¹³ C, ¹⁵ N ₂ hydrochloride | 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 640492 | 2-Nitrobenzenesulfonyl chloride- ¹³ C ₆ | 99 atom % ¹³ C |
| 493244 | Phenyl-d ₅ isocyanate | 98 atom % D |
| 603597 | Phenyl- ¹³ C ₆ isocyanate | 99 atom % ¹³ C |
| 615692 | Propionic anhydride-d ₁₀ | 98 atom % D |
| 455695 | Propylene oxide-d ₆ | 98 atom % D |
| 632341 | Suberic acid-2,2,7,7-d ₄ bis (3-sulfo-N-hydroxysuccinimide ester) disodium salt | 98 atom % D |
| 293741 | Succinic anhydride-2,2,3,3-d ₄ | 98 atom % D |
| 603902 | Succinic anhydride-1,4- ¹³ C ₂ | 99 atom % ¹³ C |

Isotopically Labeled Protected Amino Acids for Peptide Synthesis

The incorporation of stable isotope-labeled, protected amino acids into peptides via synthetic routes is important in many scientific disciplines including mass spectrometric research. ISOTEC offers a full selection of uniformly labeled, singly and doubly protected amino acids for peptide synthesis applications. In addition to being a leading manufacturer of high-purity, fully enriched protected amino acids, we have the technical expertise to produce selectively labeled, protected amino acids. For customer convenience, we provide custom packaging upon request.

Uniformly Labeled Doubly Protected Amino Acids

| Cat. No. | Name | Isotopic Purity |
|----------|---|---|
| 588407 | Boc-Glu(OBzl)- ¹³ C ₅ , ¹⁵ N | 98 atom % ¹³ C 98 atom % ¹⁵ N |
| 672866 | Boc-Thr(Bzl)-OH- ¹³ C ₄ , ¹⁵ N | NEW 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 653659 | Fmoc-Arg(Pbf)-OH- ¹³ C ₆ , ¹⁵ N ₄ | 98 atom % ¹³ C 98 atom % ¹⁵ N |
| 668753 | Fmoc-Asn(Trt)-OH- ¹³ C ₄ , ¹⁵ N ₂ | NEW 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 668745 | Fmoc-Asn(Trt)-OH- ¹⁵ N ₂ | NEW 98 atom % ¹⁵ N |
| 594075 | Fmoc-Asp(OtBu)-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 683639 | Fmoc-Asp(OtBu)-OH- ¹³ C ₄ , ¹⁵ N | NEW 98 atom % ¹³ C 98 atom % ¹⁵ N |
| 676608 | Fmoc-Cys(Trt)-OH- ¹⁵ N | NEW 98 atom % ¹⁵ N |
| 663956 | Fmoc-Gln(Trt)-OH- ¹³ C ₅ , ¹⁵ N ₂ | 98 atom % ¹³ C 98 atom % ¹⁵ N |
| 666009 | Fmoc-Glu(OtBu)-OH- ¹³ C ₅ , ¹⁵ N | 99 atom % ¹³ C 98 atom % ¹⁵ N |

Single Protected Amino Acids

| Cat. No. | Name | Isotopic Purity |
|----------|---|--|
| 486787 | Boc-Ala-OH-3,3,3-d ₃ | 99 atom % D |
| 492884 | Boc-Ala-OH- ¹² C ₃ | 99.9 atom % ¹² C |
| 486760 | Boc-Ala-OH-1- ¹³ C | 99 atom % ¹³ C |
| 605077 | Boc-Ala-OH-2- ¹³ C | 99 atom % ¹³ C |
| 603449 | Boc-Ala-OH-2- ¹³ C, ¹⁵ N | 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 492892 | Boc-Ala-OH-3- ¹³ C | 99 atom % ¹³ C |
| 586749 | Boc-Ala-OH- ¹³ C ₃ | 99 atom % ¹³ C |
| 605050 | Boc-D-Ala-OH-3- ¹³ C | 99 atom % ¹³ C |
| 485837 | Boc-Ala-OH- ¹³ C ₃ , ¹⁵ N | 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 489913 | Boc-Ala-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 579785 | Boc-Asn-OH-α- ¹⁵ N ₁ (amine- ¹⁵ N) | 98 atom % ¹⁵ N |
| 586188 | Boc-Asp-OH-3- ¹³ C | 99 atom % ¹³ C |
| 586404 | Boc-Asp-OH-4- ¹³ C | 99 atom % ¹³ C |
| 588792 | Boc-Asp-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 587702 | Boc-Gln-OH- ¹⁵ N ₂ | 98 atom % ¹⁵ N |
| 587680 | Boc-Glu-OH-1- ¹³ C | 99 atom % ¹³ C |
| 587699 | Boc-Glu-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 587710 | Boc-Gly-OH-2,2-d ₂ | 98 atom % D |
| 486698 | Boc-Gly-OH-1- ¹³ C | 99 atom % ¹³ C |
| 485780 | Boc-Gly-OH-2- ¹³ C | 99 atom % ¹³ C |

| Cat. No. | Name | Isotopic Purity |
|----------|---|---|
| 609153 | Fmoc-Glu(OtBu)-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 676969 | Fmoc-His(Trt)-OH- ¹⁵ N ₃ | NEW 98 atom % ¹⁵ N |
| 653632 | Fmoc-Lys(Boc)-OH- ¹³ C ₆ , ¹⁵ N ₂ | 98 atom % ¹³ C 98 atom % ¹⁵ N |
| 577960 | Fmoc-Lys(Boc)-OH- ¹⁵ N ₂ | 98 atom % ¹⁵ N |
| 609145 | Fmoc-Ser(tBu)-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 658928 | Fmoc-Ser(tBu)-OH- ¹³ C ₃ , ¹⁵ N | 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 658162 | Fmoc-Thr(tBu)-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 694274 | Fmoc-Thr(tBu)-OH- ¹³ C ₄ , ¹⁵ N | NEW 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 676977 | Fmoc-Trp(Boc)-OH- ¹⁵ N ₂ | NEW 98 atom % ¹⁵ N |
| 658898 | Fmoc-Tyr(tBu)-OH- ¹³ C ₉ , ¹⁵ N | 98 atom % ¹³ C 98 atom % ¹⁵ N |
| 658901 | Fmoc-Tyr(tBu)-OH- ¹⁵ N | 98 atom % ¹⁵ N |

| Cat. No. | Name | Isotopic Purity |
|----------|--|--|
| 489557 | Boc-Gly-OH-2- ¹³ C, ¹⁵ N | 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 587729 | Boc-Gly-OH-1- ¹³ C, ¹⁵ N | 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 604992 | Boc-Gly-OH- ¹³ C ₂ | 99 atom % ¹³ C |
| 587737 | Boc-Gly-OH- ¹³ C ₂ , ¹⁵ N | 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 486701 | Boc-Gly-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 615900 | Boc-Leu-OH-5,5,5-d ₃ monohydrate | 99 atom % D |
| 492930 | Boc-Leu-OH- ¹⁵ N monohydrate | 98 atom % ¹⁵ N |
| 609161 | Boc-Lys(Z)-OH-α- ¹⁵ N | 98 atom % ¹⁵ N |
| 589845 | Boc-Met-OH-1- ¹³ C | 99 atom % ¹³ C |
| 589853 | Boc-Met-OH- ¹³ C ₁ (methyl- ¹³ C) | 99 atom % ¹³ C |
| 605204 | Boc-Phe-OH-2- ¹³ C | 99 atom % ¹³ C |
| 486833 | Boc-Phe-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 591092 | Boc-Tyr-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 616222 | Boc-Val-OH-d ₃ | 98 atom % D |
| 604976 | Boc-Val-OH-1- ¹³ C | 99 atom % ¹³ C |
| 486019 | Boc-Val-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 485888 | Fmoc-Ala-OH-3,3,3-d ₃ | 99 atom % D |
| 486752 | Fmoc-Ala-OH-1- ¹³ C | 99 atom % ¹³ C |
| 605158 | Fmoc-Ala-OH-2- ¹³ C | 99 atom % ¹³ C |



Single Protected Amino Acids, continued

| Cat. No. | Name | Isotopic Purity |
|----------|---|--|
| 489956 | Fmoc-Ala-OH-3- ¹³ C | 99 atom % ¹³ C |
| 605131 | Fmoc-Ala-OH- ¹³ C ₃ | 99 atom % ¹³ C |
| 667064 | Fmoc-Ala-OH, ¹³ C ₃ , ¹⁵ N monohydrate NEW | 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 489905 | Fmoc-Ala-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 609137 | Fmoc-Asn-OH-α- ¹⁵ N ₁ (amine- ¹⁵ N) | 98 atom % ¹⁵ N |
| 579890 | Fmoc-Asn-OH- ¹⁵ N ₂ | 98 atom % ¹⁵ N |
| 588628 | Fmoc-Asp-OH-1- ¹³ C | 99 atom % ¹³ C |
| 594695 | Fmoc-Asp-OH-2- ¹³ C | 99 atom % ¹³ C |
| 605263 | Fmoc-Asp-OH-4- ¹³ C | 99 atom % ¹³ C |
| 492906 | Fmoc-Asp-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 490008 | Fmoc-Glu-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 485772 | Fmoc-Gly-OH-2,2-d ₂ | 98 atom % D |
| 605182 | Fmoc-Gly-OH-1- ¹³ C | 99 atom % ¹³ C |
| 492698 | Fmoc-Gly-OH-1- ¹³ C, ¹⁵ N | 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 489549 | Fmoc-Gly-OH-2- ¹³ C | 99 atom % ¹³ C |
| 603457 | Fmoc-Gly-OH-2- ¹³ C, ¹⁵ N | 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 587745 | Fmoc-Gly-OH- ¹³ C ₂ | 99 atom % ¹³ C |
| 489530 | Fmoc-Gly-OH- ¹³ C ₂ , ¹⁵ N | 99 atom % ¹³ C 98 atom % ¹⁵ N |
| 485756 | Fmoc-Gly-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 597228 | Fmoc-Ile-OH- ¹³ C ₆ , ¹⁵ N | 98 atom % ¹³ C 98 atom % ¹⁵ N |
| 578622 | Fmoc-Ile-OH- ¹⁵ N | 98 atom % ¹⁵ N |

| Cat. No. | Name | Isotopic Purity |
|----------|--|--|
| 615943 | Fmoc-Leu-OH-5,5,5-d ₃ | 99 atom % D |
| 485934 | Fmoc-Leu-OH-1- ¹³ C | 99 atom % ¹³ C |
| 485950 | Fmoc-Leu-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 605115 | Fmoc-Met-OH-1- ¹³ C | 99 atom % ¹³ C |
| 653640 | Fmoc-Met-OH- ¹³ C ₅ , ¹⁵ N | 98 atom % ¹³ C 98 atom % ¹⁵ N |
| 609196 | Fmoc-Met-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 615994 | Fmoc-Phe-OH- <i>phenyl</i> -d ₅ -2,3,3-d ₃ | 98 atom % D |
| 492965 | Fmoc-Phe-OH-2- ¹³ C | 99 atom % ¹³ C |
| 651443 | Fmoc-Phe-OH- ¹³ C ₉ , ¹⁵ N | 98 atom % ¹³ C 98 atom % ¹⁵ N |
| 609072 | Fmoc-Phe-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 651451 | Fmoc-Pro-OH- ¹³ C ₉ , ¹⁵ N | 98 atom % ¹³ C 98 atom % ¹⁵ N |
| 589519 | Fmoc-Pro-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 653624 | Fmoc-Tyr-OH- ¹⁵ N | 98 atom % ¹⁵ N |
| 616087 | Fmoc-Val-OH-d ₈ | 98 atom % D |
| 485993 | Fmoc-Val-OH-1- ¹³ C | 99 atom % ¹³ C |
| 642886 | Fmoc-Val-OH- ¹³ C ₅ , ¹⁵ N | 98 atom % ¹³ C 98 atom % ¹⁵ N |
| 486000 | Fmoc-Val-OH- ¹⁵ N | 98 atom % ¹⁵ N |

Proteome Profiler ¹⁸O Enzymatic Labeling Kit

Trypsin-mediated incorporation of ¹⁸O remains an important technique for the exogenous isotopic enrichment of proteins for quantitative proteomics. Two ¹⁸O atoms are introduced into the carboxy terminus of protein fragments during proteolytic cleavage in heavy water. The quantification of protein samples is achieved by combining natural abundance ¹⁶O fragments and ¹⁸O labeled peptide fragments then subjecting the mixture to mass spectrometric analysis to determine the ratio of ¹⁶O/¹⁸O labeled peak pairs ⁽¹⁻²⁾. ¹⁸O enzymatic labeling has gained popularity in the examination of differential protein expression in pharmacological and cancer research ⁽³⁻⁴⁾. For this application, the ¹⁸O Proteome Profiler Kit ensures a reliable and effective means of isotope incorporation. The kit includes material sufficient to perform 12 protein quantification experiments. We also offer additional products for ¹⁸O-based proteomics studies.

Enzymatic Labeling Products

| Cat. No. | Name | Isotopic Purity |
|----------|---|---------------------------|
| P3623 | ¹⁸ O Proteome Profiler Kit NEW | |
| 487090 | Water- ¹⁸ O | 99 atom % ¹⁸ O |
| 329878 | Water- ¹⁸ O | 97 atom % ¹⁸ O |

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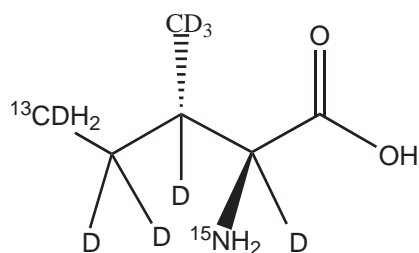
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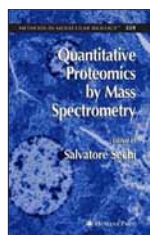
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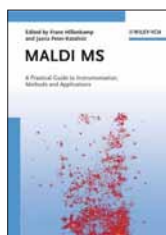
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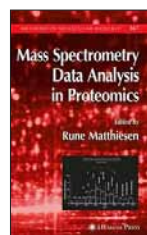
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