

# MASS SPECTROMETRY



## Components

Note: Components included in this complete kit may also be included in the protein or peptide-specific kits. Please review the following pages for protocols and sample spectra.

## Features & Benefits:

- **Convenient Kit Packaging** – No weighing required: Standards and matrices are pre-weighed to save time and limit waste.
- **Flexible** – Standards can be mixed and matched to bracket any desired mass range between 700 and 67,000 Da.
- **Stringent Quality Control Measures** – Tested and pre-qualified materials ensure an absence of impurities and reduced contaminant interference.
- **Complete Kit for All Applications: Calibration, Tuning and Sensitivity** – A combination of peptides provides accurate and precise calibration across the typical mass range of tryptic digestion fragments (800-3,000 Da) in reflectron mode. A mixture of proteins allows for calibration over a wide mass range in linear mode, from 5,000 up to 67,000 Da depending on the proteins combined.

## ProteoMass™ Peptide & Protein MALDI-MS Calibration Kit

For the calibration, tuning and sensitivity testing of MALDI-MS instruments

Mass range of standards: 757 Da to 66,430 Da

This kit is appropriate for evaluating complex mixtures of proteins and peptides that span a broad range of molecular weights. Featuring a conveniently-packed selection of peptides, proteins, solvents, and matrices qualified for MALDI-MS applications, this kit is applicable for tuning, testing, or routine calibration of either new or existing MALDI equipment. The controls included are commonly used in protein/peptide mass spectrometry, and offer flexibility for either internal or external calibration. To ensure accurate spectra, all components are tested to guarantee freedom from significant amounts of interfering contaminants. Specifically-selected packaging materials eliminate potential introduction of harmful metal ions, which lessen ionization efficiency and contribute to metal adducts. In addition, ready-to-use corrosive solvents minimize handling and improve safety. Included application information helps to select the optimal sample preparation method.

## Matrices

Each matrix is provided in four 2 ml amber vials containing 10 mg each.

Product Description	Common Name	Total/Kit
$\alpha$ -Cyano-4-hydroxycinnamic acid	$\alpha$ -cyano CHCA	40 mg
3,5-Dimethoxy-4-hydroxycinnamic acid	Sinapinic acid	40 mg

## Standard Peptides and Proteins

Standard peptides and proteins are supplied in 1.5 ml clear vials, each containing 10 nmoles of standard.

Product Description	(M+H) <sup>+</sup> *	Total/Kit
Bradykinin Fragment 1-7	757.3997(monoisotopic)	10 nmoles
Angiotensin II (human)	1,046.5423(monoisotopic)	10 nmoles
P <sub>14</sub> R (synthetic peptide)	1,533.8582(monoisotopic)	10 nmoles
ACTH Fragment 18-39 (human)	2,465.1989(monoisotopic)	10 nmoles
Insulin oxidized B chain (bovine)	3,494.6513(monoisotopic)	10 nmoles
Insulin (bovine)	5,730.6087(monoisotopic) 5,734.51 (avg)	10 nmoles
Cytochrome c (equine)	12,361.96 (avg)	10 nmoles
Apomyoglobin (equine)	16,952.27 (avg)	10 nmoles
Aldolase (rabbit muscle)	39,212.28 (avg)	10 nmoles
Albumin (bovine serum) - BSA	66,430.09 (avg)	10 nmoles

\*Masses were calculated based on NCBI sequences using NIST standard atomic weights and isotopic masses.

## Solvents

Solvents are supplied in high density polyethylene bottles.

Product Description	Units/Kit	Amt/Unit	Total/Kit
0.1% Trifluoroacetic acid (TFA) Solution	1	30 ml	30 ml
Acetonitrile (ACN)	1	30 ml	30 ml
1% Trifluoroacetic acid (TFA) Solution	1	4 ml	4 ml

Product Code	Description	Size
<a href="#">MS-CAL1</a>	ProteoMass Peptide & Protein MALDI-MS Calibration Kit	1 kit
<a href="#">C 8982</a>	$\alpha$ -Cyano-4-hydroxycinnamic acid	10 x 10 mg
<a href="#">S 8313</a>	3,5-Dimethoxy-4-hydroxycinnamic acid	10 x 10 mg
<a href="#">B 4181</a>	Bradykinin Fragment 1-7	5 x 10 nmoles
<a href="#">A 8846</a>	Angiotensin II (human)	5 x 10 nmoles
<a href="#">P 2613</a>	P <sub>14</sub> R (synthetic peptide)	5 x 10 nmoles
<a href="#">A 8346</a>	ACTH Fragment 18-39 (human)	5 x 10 nmoles
<a href="#">I 6154</a>	Insulin oxidized B chain (bovine)	5 x 10 nmoles
<a href="#">I 6279</a>	Insulin (bovine)	5 x 10 nmoles
<a href="#">C 8857</a>	Cytochrome c (equine)	5 x 10 nmoles
<a href="#">A 8971</a>	Apomyoglobin (equine)	5 x 10 nmoles
<a href="#">A 9096</a>	Aldolase (rabbit muscle)	5 x 10 nmoles
<a href="#">A 8471</a>	Albumin (bovine serum) - BSA	5 x 10 nmoles

# MASS SPECTROMETRY

## ProteoMass™ Peptide MALDI-MS Calibration Kit

For the calibration, tuning and sensitivity testing of MALDI-MS instruments

Mass range of standards: 757 Da to 3,494 Da

This kit features a conveniently-packed selection of peptides, solvents and matrices qualified for MALDI-MS applications, and is applicable for tuning, testing or routine calibration of either new or existing MALDI equipment. The controls included provide accurate calibration over the typical mass range of tryptic digestion fragments (800-3000 Da) in reflectron mode, and offer flexibility in range choice for either internal or external calibration. The controls Angiotensin II and P<sub>14</sub>R provide multiple post-source decay (PSD) fragments for calibration of PSD data. To ensure accurate spectra, all components are tested to guarantee freedom from significant amounts of interfering contaminants. Specifically-selected packaging materials eliminate potential introduction of harmful metal ions which lessen ionization efficiency and contribute to metal adducts. In addition, ready-to-use corrosive solvents minimize handling and improve safety. Application information included helps to select the optimal sample preparation method.

### Components

#### Matrix

The matrix is provided in eight 2 ml amber vials containing 10 mg each.

Product Description	Common Name	Units/Kit	Amt/Unit	Total/Kit
$\alpha$ -Cyano-4-hydroxycinnamic acid	$\alpha$ -cyano CHCA	8	10 mg	80 mg

#### Standard Peptides

Two of each standard peptide are supplied in 1.5 ml clear tubes, each containing 10 nmoles of standard.

Product Description	(M+H) <sup>+</sup> *	Units/Kit	Amt/Unit	Total/Kit
Bradykinin Fragment 1-7 (monoisotopic)	757.3997	2	10 nmoles	20 nmoles
Angiotensin II (human)	1,046.5423 (monoisotopic)	2	10 nmoles	20 nmoles
P <sub>14</sub> R (synthetic peptide)	1,533.8582 (monoisotopic)	2	10 nmoles	20 nmoles
ACTH Fragment 18-39 (human)	2,465.1989 (monoisotopic)	2	10 nmoles	20 nmoles
Insulin oxidized B chain (bovine)	3,494.6513 (monoisotopic)	2	10 nmoles	20 nmoles

\*Masses were calculated based on NCBI sequences using NIST standard atomic weights and isotopic masses.

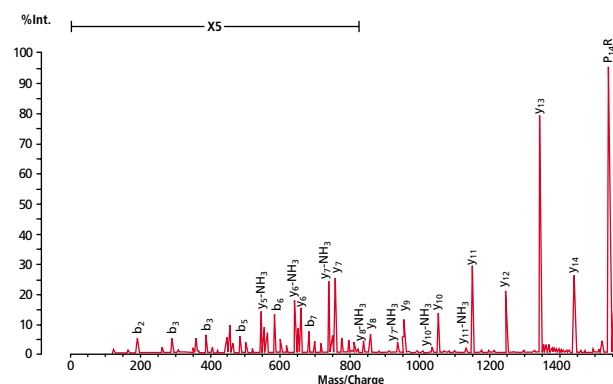
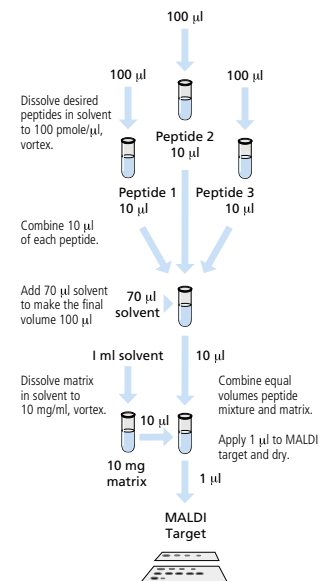
#### Solvents

Solvents are supplied in high density polyethylene bottles.

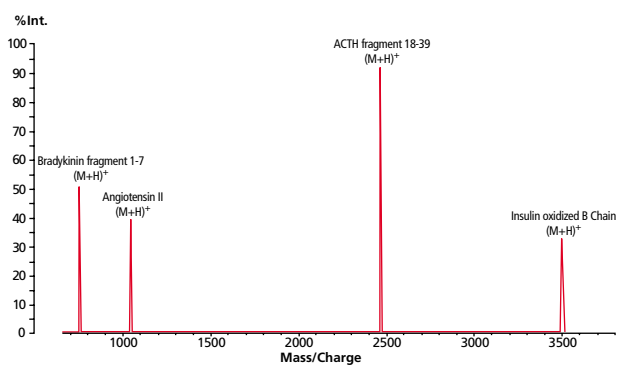
Product Description	Units/Kit	Amt/Unit	Total/Kit
0.1% Trifluoroacetic acid (TFA) Solution	1	30 ml	30 ml
Acetonitrile (ACN)	1	30 ml	30 ml
1% Trifluoroacetic acid (TFA) Solution	1	4 ml	4 ml



### Protocol for Calibration



Post source decay analysis of P<sub>14</sub>R using  $\alpha$ -cyano-4-hydroxycinnamic acid as the MALDI matrix. Data was acquired on a Kratos Analytical AXIMA-CFR system™ in reflectron positive ion mode.



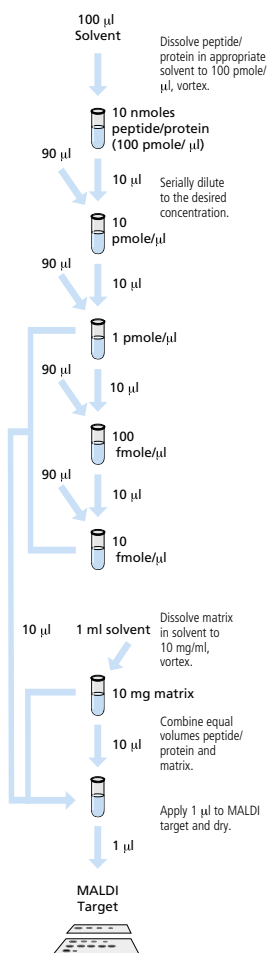
MALDI mass spectrum of a peptide calibration solution containing 1.5  $\mu$ M Bradykinin fragment 1-7, 1.0  $\mu$ M Angiotensin II, 0.5  $\mu$ M ACTH fragment 18-39, and 2.0  $\mu$ M Insulin oxidized B chain. A 10  $\mu$ l aliquot of the peptide solution in 0.1% TFA was mixed with 10  $\mu$ l of a 10 mg/ml  $\alpha$ -cyano-4-hydroxycinnamic acid in 50% ACN, 0.05% TFA. 0.8  $\mu$ l of the resulting solution was spotted onto the MALDI target. Analysis was done using a Kratos Kompact SEQ system™ in the linear positive ion mode.

Product Code	Description	Size
MS-CAL2	ProteoMass Peptide MALDI-MS Calibration Kit	1 kit

# MASS SPECTROMETRY



## Protocol for Sensitivity



## ProteoMass™ Protein MALDI-MS Calibration Kit

For the calibration, tuning and sensitivity testing of MALDI-MS instruments

Mass range of standards: 5,730 Da to 66,430 Da

This kit features a conveniently-packed selection of proteins, solvents, and matrices qualified for MALDI-MS applications and is applicable for tuning, testing or routine calibration of either new or existing MALDI equipment. The controls enable calibration over the typical protein mass range of 5,730 Da to 66,430 Da, and offer flexibility in range choice for either internal or external calibration. To ensure accurate spectra, all components are tested to guarantee freedom from significant amounts of interfering contaminants. Specifically-selected packaging materials eliminate potential introduction of harmful metal ions which lessen ionization efficiency and contribute to metal adducts. In addition, ready-to-use corrosive solvents minimize handling and improve safety. Application information included helps to select the optimal sample preparation method.

## Components

### Matrix

The matrix is provided in eight 2 ml amber vials containing 10 mg each.

Product Description	Common Name	Units/Kit	Amt/Unit	Total/Kit
3,5-Dimethoxy-4-hydroxycinnamic acid	Sinapinic acid	8	10 mg	80 mg

### Standard Proteins

Two of each standard protein are supplied in 1.5 ml clear tubes, each containing 10 nmoles of standard.

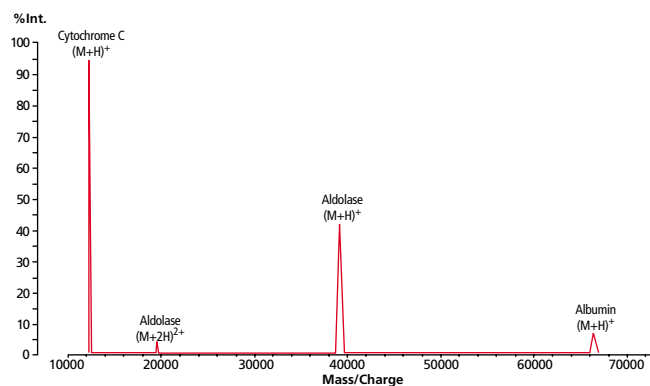
Product Description	(M+H) <sup>+</sup>	Units/Kit	Amt/Unit	Total/Kit
Insulin (bovine)	5,730.6087 (monoisotopic) 5,734.51 (avg)	2	10 nmoles	20 nmoles
Cytochrome c (equine)	12,361.96 (avg)	2	10 nmoles	20 nmoles
Apomyoglobin (equine)	16,952.27 (avg)	2	10 nmoles	20 nmoles
Aldolase (rabbit muscle)	39,212.28 (avg)	2	10 nmoles	20 nmoles
Albumin (bovine serum) - BSA	66,430.09 (avg)	2	10 nmoles	20 nmoles

\*Masses were calculated based on NCBI sequences using NIST standard atomic weights and isotopic masses.

### Solvents

Solvents are supplied in high density polyethylene bottles.

Product Description	Units/Kit	Amt/Unit	Total/Kit
0.1% Trifluoroacetic acid (TFA) Solution	1	30 ml	30 ml
Acetonitrile (ACN)	1	30 ml	30 ml
1% Trifluoroacetic acid (TFA) Solution	1	4 ml	4 ml



MALDI mass spectrum of a protein calibration solution containing 1.0 µM Cytochrome C, 2.0 µM Aldolase, and 10 µM Albumin. A 10 µl aliquot of the protein solution in 0.1% TFA was mixed with 10 µl of a 10 mg/ml sinapinic acid solution in 70% ACN, 0.03% TFA. 0.8 µl of the resulting solution was spotted onto the MALDI target. Analysis was done using a Kratos Kompact SEQ system in the linear positive ion mode.

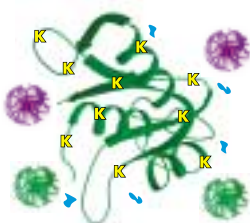
Product Code	Description	Size
<a href="#">MS-CAL3</a>	ProteoMass Protein MALDI-MS Calibration Kit	1 kit

# MASS SPECTROMETRY

## MALDI Matrices

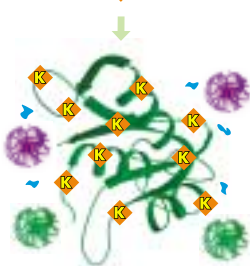
Analyte	Recommended Matrix	Acronym	Product Code	Size
<b>Peptide/Protein</b>				
Mass < 10kDa	$\alpha$ -Cyano-4-hydroxycinnamic acid	CHCA	<a href="#">C 8982</a>	10 x 10 mg
Mass > 10 kDa	Sinapic acid	SA	<a href="#">S 8313</a>	10 x 10 mg
	2-(4-Hydroxyphenylazo)benzoic acid	HABA	<a href="#">54793</a>	1 g, 5 g
IR-Laser	Succinic acid		<a href="#">14078</a>	1 g, 5 g
UV-Laser	2,6-Dihydroxyacetophenone		<a href="#">37468</a>	1 g, 5 g
UV-Laser	Ferulic acid		<a href="#">46278</a>	1 g, 5 g
UV-Laser	Caffeic acid		<a href="#">60018</a>	1 g, 5 g
Liquid matrix	Glycerol		<a href="#">49771</a>	1 ml, 5 ml
Liquid Matrix	4-Nitroaniline		<a href="#">72681</a>	250 mg, 1 g
<b>Oligonucleotide</b>				
Mass < 3.5 kDa	2,4,6-Trihydroxyacetophenone	THAP	<a href="#">91928</a>	1 g, 5 g
Mass > 3.5 kDa	3-Hydroxypicolinic acid	HPA	<a href="#">56197</a>	1 g, 250 mg
	Anthranilic acid		<a href="#">10678</a>	1 g
	Nicotinic acid		<a href="#">72311</a>	250 mg, 1 g
<b>Synthetic Polymer</b>				
Non-polar	Trans-3-indoleacrylic acid	IAA	<a href="#">57288</a>	1 g, 5 g
	Dithranol	DIT	<a href="#">10608</a>	1 g, 5 g
Polar	2,5-Dihydroxybenzoic acid	DHB	<a href="#">85707</a>	250 mg, 1 g
IR-Laser	Succinic acid		<a href="#">14078</a>	1 g, 5 g
<b>Organic Molecules</b>				
	2,5-Dihydroxybenzoic acid	DHB	<a href="#">85707</a>	250 mg, 1 g
	Isovanillin		<a href="#">59927</a>	1 g, 5 g
<b>Carbohydrates</b>				
	2,5-Dihydroxybenzoic acid	DHB	<a href="#">85707</a>	250 mg, 1 g
	$\alpha$ -Cyano-4-hydroxycinnamic acid	CHCA	<a href="#">C 8982</a>	10 x 10 mg
	3-Aminoquinoline		<a href="#">07336</a>	1 g, 5 g
Acidic	2,4,6-Trihydroxyacetophenone	THAP	<a href="#">91928</a>	1 g, 5 g
<b>Lipids</b>				
	Dithranol	DIT	<a href="#">10608</a>	1 g, 5 g
<b>Dendrimers</b>				
	Sinapic acid	SA	<a href="#">S 8313</a>	10 x 10 mg
	Dithranol	DIT	<a href="#">10608</a>	1 g, 5 g
<b>Fullerenes</b>				
	Sinapic acid	SA	<a href="#">S 8313</a>	10 x 10 mg
<b>Inorganic molecules</b>				
	T-2-(3-(4-t-Butyl-phenyl)-2-methyl-2-propenylidene)malononitrile	DCTB	<a href="#">87884</a>	250 mg, 1 g
<b>Oligosaccharide</b>				
	1-isoquinolinol		<a href="#">55433</a>	1 g, 5 g

# MASS SPECTROMETRY

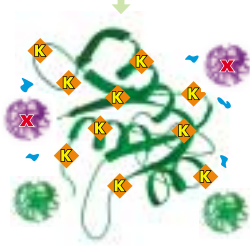


Porcine trypsin contains 10 Lysine (K) residues; each represents a potential site for autodigestion. Chymotrypsin and other contaminant proteins are often present as well.

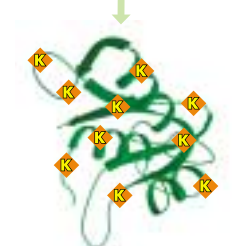
◆ Total dimethylation of the  $\epsilon$ -amino groups of lysine protects trypsin from autodigestion.



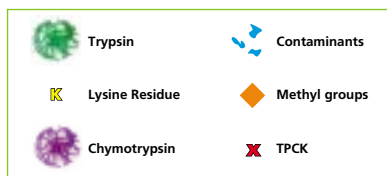
✗ TPCK binds irreversibly to the active sites of any chymotrypsin molecules present, rendering them inactive.



✗ Affinity purification removes contaminants, improving activity and preventing ambiguous MALDI spectra.



Proteomics Grade Trypsin is dimethylated at each Lysine (K) residue, preventing autodigestion. Any residual inactivated Chymotrypsin molecules and other minor contaminants have been removed.



## Proteomics Grade Trypsin

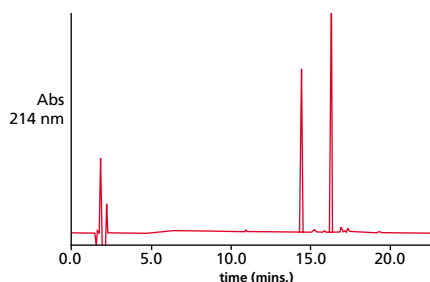
For the production of tryptic protein digests for MALDI-MS analysis

Proteomics Grade Trypsin has been extensively purified from porcine pancreas to accurately and precisely hydrolyze proteins at the carboxylic side of arginine and lysine residues. This very stable trypsin has been reductively methylated to reduce autolysis, and TPCK (N-Tosyl-L-phenylalanine chloromethyl ketone) treated to inhibit chymotryptic activity. Further purification by affinity chromatography and lyophilization from a dilute acid yields a highly purified, high specific-activity trypsin suitable for proteomics research. Proteomics Grade Trypsin provides excellent performance in either solution or in-gel tryptic digestions. The product is conveniently packed in five 20  $\mu$ g vials to help ensure fresh enzyme is available for each use.



### Features & Benefits

- **Highly purified** – Eliminates contaminant peaks and non-tryptic fragments from spectra
- **Stabilized form retains high specific activity** – Complete digest with less protease for economy and confidence in spectra
- **Application document included with in-gel digest example** – Save time in methods training and development
- **Convenient packaging** – Save time and reduce waste by using only amount needed



Retention Time (min)	Mass (Da)*	Fragment
14.40	858.4	Gly(23)-Lys(29)
16.26	2583.0	Phe(1)-Arg(22)

The hydrophilic amino acid Ala (30) co-elutes with the buffer salts in the injection peak. During an 18-hour digestion only the expected peptides were generated with no indication of other proteolytic activity. Under these conditions the cleavage of the substrate peptide is complete in less than 5 minutes.

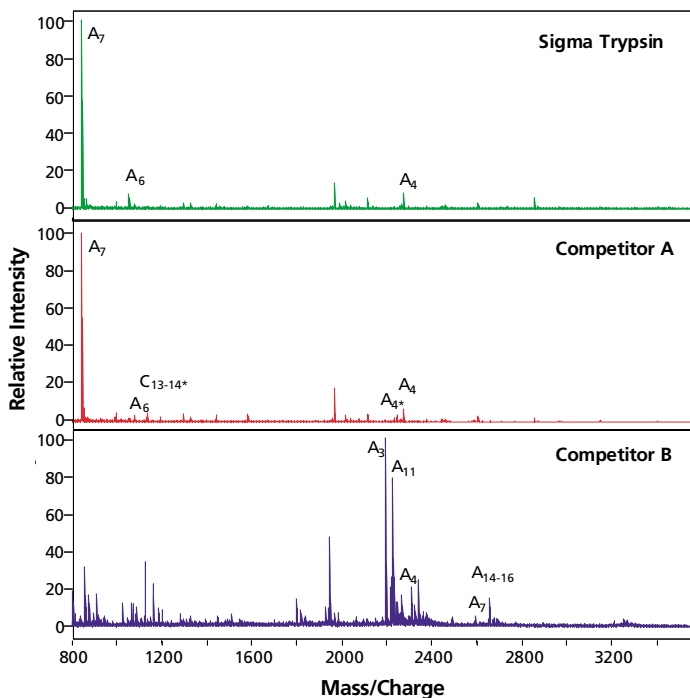
\*Determined by Electrospray MS

*Trypsin Solution Digestion. The insulin oxidized B chain (100  $\mu$ g) was digested with 2  $\mu$ g of Proteomics Grade Trypsin for 18 hours at 37 °C in 100  $\mu$ l of 100 mM Tris-HCl, pH 8.5. A 10  $\mu$ g aliquot was separated on a Supelco Discovery™ C18 column (15 cm x 4.6 mm, 5 micron, Product Code 504955), using a 20 minute linear gradient from 5-50% B at 1 ml/min with UV detection at 214 nm. Solvent A: 0.1% (v/v) TFA in water. Solvent B: 0.08% (v/v) TFA in acetonitrile.*

Product Code	Description	Size
<a href="#">T 6567</a>	Proteomics Grade Trypsin	5 x 20 $\mu$ g

# MASS SPECTROMETRY

## MALDI Analysis of Autolysis Products from Commercial Trypsins



### Legend for Competitor A and Sigma Trypsin (porcine source)

Autolysis Fragment	Amino Acids	(M+H) <sup>+</sup>	Form
A <sub>4</sub> *	50-69	2211.1046	Unmethylated
A <sub>4</sub>	50-69	2239.1359	Dimethylated
A <sub>6</sub>	90-99	1045.5642	N/A
A <sub>7</sub>	100-107	842.5100	N/A

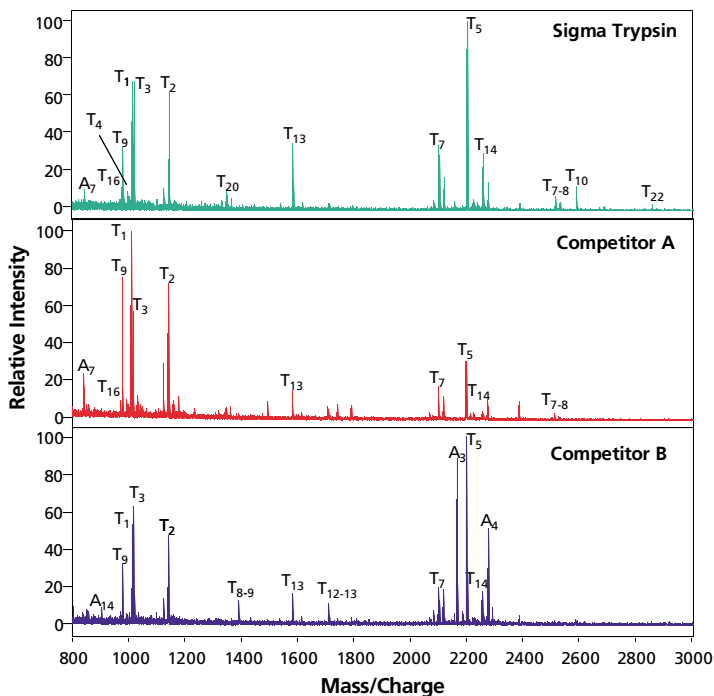
Chymotryptic Fragment	Amino Acids	(M+H) <sup>+</sup>	Form
C <sub>13-14</sub> *	207-215	1125.5277	Unmethylated

### Legend for Competitor B (bovine source)

Autolysis Fragment	Amino Acids	(M+H) <sup>+</sup>
A <sub>3</sub>	50-69	2163.0570
A <sub>4</sub>	70-89	2273.1600
A <sub>7</sub>	100-125	2550.2332
A <sub>11</sub>	150-170	2192.9871
A <sub>14-16</sub>	201-223	2612.3421

Sigma's Proteomics Grade Trypsin produces fewer autolytic fragments than the competition. Other trypsin contain higher levels of autolytic fragments, as well as chymotryptic fragments, both of which can lead to ambiguous MALDI spectra or poor ionization of tryptic peptides. To compare brands, an autodigestion experiment was performed. In each instance, 5 µg of Trypsin was incubated without an alternative substrate in 100 µl of 100 mM ammonium bicarbonate at 37 °C overnight. MALDI analysis was performed in the reflectron positive ion mode using α-cyano-4-hydroxycinnamic acid (10 mg/mL in 70% ACN, 0.03% TFA) as the matrix. The sample was mixed 1:1 with the matrix and 1 µl was spotted onto the MALDI target.

## In Gel Trypsin Digestion of 20 pmoles of Carbonic Anhydrase II



### Legend

Tryptic Fragment	Amino Acids	(M+H) <sup>+</sup>
T <sub>1</sub>	1-8	1013.4593
T <sub>2</sub>	9-17	1140.5451
T <sub>3</sub>	18-26	1018.4958
T <sub>4</sub>	27-35	1001.5268
T <sub>5</sub>	36-56	2198.2185
T <sub>7</sub>	58-75	2098.8776
T <sub>9</sub>	80-88	979.4849
T <sub>10</sub>	89-110	2582.1925
T <sub>13</sub>	113-125	1581.8178
T <sub>14</sub>	126-147	2253.1556
T <sub>16</sub>	158-166	973.5570
T <sub>20</sub>	212-223	1346.6990
T <sub>22</sub>	226-250	2852.4769

### Missed Cleavages

T <sub>7-8</sub>	58-79	2510.1622
T <sub>8-9</sub>	76-88	1390.7694
T <sub>12-13</sub>	112-125	1709.9128

Sigma's Proteomics Grade Trypsin leads to greater sequence coverage than other Trypsins. To compare trypsin from Sigma and two other suppliers, a sequence coverage experiment was performed. In each instance, 20 pmoles of carbonic anhydrase was run on a 10% tris-glycine SDS-PAGE gel. The gel was stained with EZBlue Gel Staining Reagent (G 1041). Following destaining, the bands were excised and tryptically digested with the ProteoProfile™ Trypsin In-Gel Digest Kit (PP 0100). MALDI analysis was performed in the reflectron positive ion mode using α-cyano-4-hydroxycinnamic acid (10 mg/mL in 70% ACN, 0.03% TFA) as the matrix. The sample was mixed 1:1 with the matrix and 1 µL was spotted onto the MALDI target. Thirteen tryptic fragments (up to nearly 2,900 M+H<sup>+</sup>) of carbonic anhydrase and one autolytic fragment were identified using Sigma Proteomics Grade Trypsin; only ten tryptic fragments and one autolytic fragment were identified using competitor A's Trypsin, and ten tryptic fragments and three autolytic fragments were identified using competitor B's Trypsin.

# MASS SPECTROMETRY

## Trypsin Profile IGD Kit

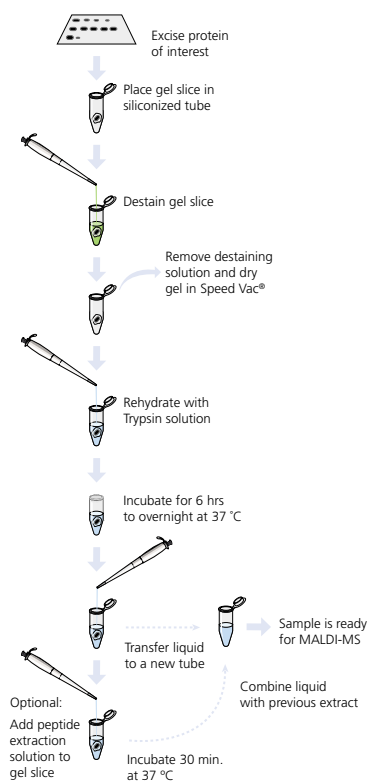


In-gel tryptic digests of 1D or 2D acrylamide gels are commonly used in proteomics to identify unknown proteins of interest. The unknown proteins are excised from the gel, digested with trypsin then analyzed via MALDI or HPLC-MS with subsequent database searching. Sigma's Trypsin IGD Kit provides a quick and easy method for the digestion of up to 100 samples.

Trypsin IGD Kit contains Proteomics Grade Trypsin and is optimized for polyacrylamide gels stained with Coomassie or SYPRO® Orange or Ruby dyes. If silver stained gels are used, a different destaining step is required (ProteoSilver™ Plus, Silver staining kit is recommended, Product Code [PROT-SIL2](#)). A destaining solution for silver stained gels is included in the ProteoSilver Plus kit and the gel pieces should be destained according to the included protocol.

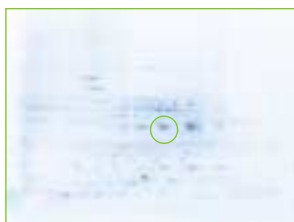
### Components

Proteomics Grade Trypsin
Destaining Solution
Trypsin Reaction Buffer
Biotech Grade Acetonitrile
Trypsin Solubilization Reagent
Peptide Extraction Solution

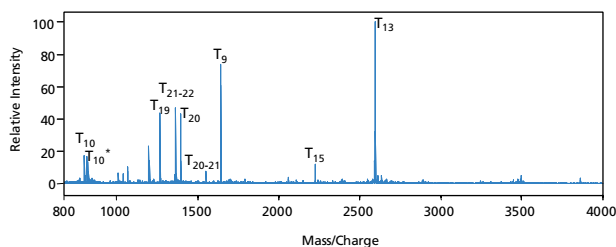


### Features & Benefits

- **Includes highly purified Proteomics Grade Trypsin** – Trypsin displays only minimal autolytic activity and no chymotryptic activity, which simplifies resulting peptide analysis
- **Detailed protocol** – Easy to follow protocol simplifies the process
- **Works with Coomassie, SYPRO Orange, SYPRO Ruby or ProteoSilver Stained gels** – Allows user a choice of staining methods
- **Resulting samples are suitable for analysis by MALDI-MS** – No further processing needed, saves time



A solution of 2.5 mg/ml *E. Coli* in Cellular and Organelle Membrane Solubilizing Reagent of ProteoPrep™ Total Extraction Sample Kit (Product Code [Prot-Tot](#)) was sonicated, then reduced with tributyl phosphine and alkylated with iodoacetamide (Product Code [PROTRA](#)). 109 µg of protein was loaded on a 7 cm, pH 4-7 IPG strip (Product Code [I2906](#)), focused for 50,000 volt hours, then run on a 4-20% Tris-Glycine SDS PAGE gel at 150 volts for 70 min. The gel was then stained with EZBlue ([G 1041](#)). A random spot was then excised and tryptically digested using the ProteoProfile Trypsin In-Gel Digest Kit (Product Code [PP 0100](#)).



The sample was desalted using a ZipTip™ C18 pipette tip from Millipore and eluted directly onto the MALDI target using the MALDI matrix ( $\alpha$ -cyano-4-hydroxycinnamic acid, 10 mg/ml in 70% ACN, 0.03% TFA). MALDI analysis was performed in the reflection positive ion mode. The resulting monoisotopic masses were searched against the NCBI database at a tolerance of 150 ppm. The protein was identified as outer membrane protein 3a from *E. Coli* with the matched peptides providing 30% sequence coverage.

Tryptic Fragment	Amino Acid	(M+H) <sup>+</sup>
T <sub>9</sub>	104-117	1654.8328
T <sub>10</sub>	118-124	818.4347
T <sub>10</sub> <sup>*</sup>	118-124 oxidized	834.4296
T <sub>13</sub>	135-159	2601.2949
T <sub>15</sub>	191-213	2232.1665
T <sub>19</sub>	252-263	1280.6487
T <sub>20</sub>	264-276	1409.6661
Missing Cleavages	Amino Acid	(M+H) <sup>+</sup>
T <sub>20-21</sub>	264-277	1565.7572
T <sub>21-22</sub>	277-288	1378.7694

Product Code	Description	Size
<a href="#">PP0100</a>	Trypsin Profile IGD Kit	1 kit

# MASS SPECTROMETRY

## Protease Profiler Kit

Identify and characterize your unknown proteins with accuracy and convenience using the Protease Profiler Kit.

Proteolytic digestion of an unknown sample protein is often used to determine or confirm its identity. Proteases with specific, optimal cleavage sites generate predictable, consistent peptide products, simplifying data interpretation. Trypsin is recognized as the most commonly used protease for protein identification — given a favorable primary structure and tertiary conformation, trypsin is an excellent choice. In some cases, trypsin may cleave an active site, or cleavage sites may be inaccessible; an alternative protease is needed. The Protease Profiler Kit provides four excellent alternative proteases, in addition to Proteomics Grade Trypsin, an enzyme solubilization reagent and enzyme reaction buffer. The Profiler Kit enables optimized, convenient digestion of target proteins with a variety of prequalified, well-established proteases.

### The Protease Profiler Kit is used for:

- **Characterization of protein structure:** Explore the primary structure of your protein of interest through digestions with enzymes of distinct specificities.
- **Double Enzymatic Digestion:** An initial digestion may result in peptide fragments greater than 5,000 Daltons, often not detected by MS. A second digestion using a protease with different specificity results in cleavage of the large peptides, generating smaller peptides suitable for MS.
- **Exploration of alternative proteases:** Trypsin is incompatible with many protein samples; find the protease best suited to your protein of interest and improve the quality of your data.
- **In-Solution or In-gel Digestion:** Optimized protocols and reagents for both digest conditions are provided, enabling flexibility in your analysis.

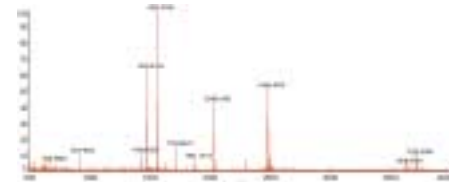
Proteases are also available individually.

Enzyme	Specificity	Optimal pH	Average MW
Proteomics Grade Trypsin	Carboxyl side of arginine and lysine residues	pH 8.0	23.3 kDa
Asp-N	Amine side of Aspartic and Cysteic acid residues	pH 6.0 - 8.5	24.5 kDa
Glu-C	Carboxyl side of glytanyl and aspartyl residues	pH 4.0 - 7.8	29.0 kDa
Lys-C	Carboxyl side of Lysine residues	pH 8.5	28.0 kDa
Arg-C	Carboxyl side of Arginine residues, as well as Lys-Lys	pH 7.5 - 8.5	26.5 kDa

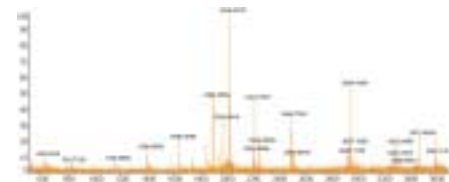
Product Code	Description	Size
<a href="#">PP 0500</a>	Protease Profiler Kit	1 kit
<a href="#">T 6567</a>	Proteomics Grade Trypsin	5 x 20 µg
<a href="#">P 3303</a>	Asp-N Protease	2 µg
<a href="#">P 6181</a>	Glu-C Protease	25 µg
<a href="#">P 3428</a>	Lys-C Protease	5 µg
<a href="#">P 6056</a>	Arg-C Protease	5 µg

Components	
Asp-N	2 µg
Glu-C	25 µg
Lys-C	5 µg
Arg-C	5 µg
Proteomics Grade Trypsin	20 µg
Enzyme Solubilization Reagent	1 ml
Enzyme Reaction Buffer	25 ml

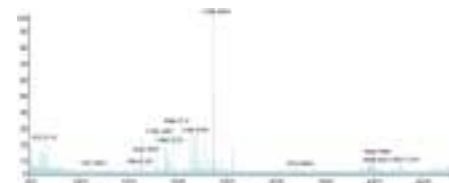
### Proteomics Grade Trypsin



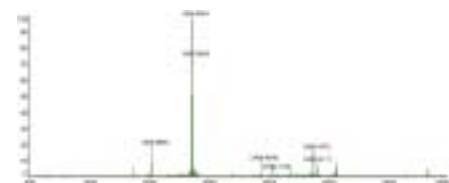
### Lys-C



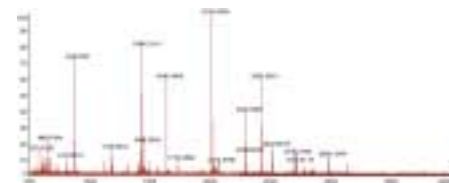
### Asp-N



### Glu-C

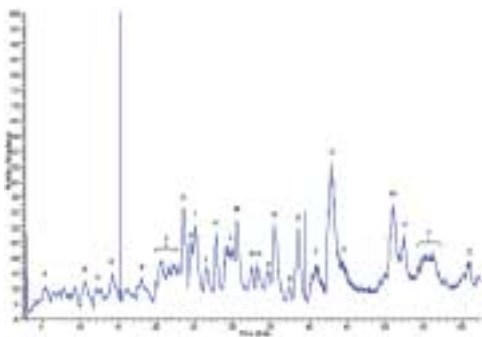


### Arg-C



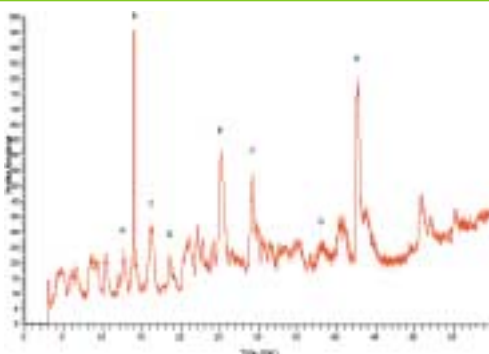
**Explore alternative proteases** Each enzyme creates a distinct peptide map; choose the digest that best allows you to characterize your protein of interest. A 2 mg/ml solution of Bovine Serum Albumin was reduced with TBP and alkylated with iodoacetamide (Product Code [PROT-RA](#)). The BSA was digested with Proteomics Grade Trypsin, Lys-C, Asp-N, Glu-C, and Arg-C. Samples were incubated for 17 hours at 37 °C. Samples were spotted in 10 mg/ml CHCA in 70% acetonitrile 0.03% TFA. MALDI-MS was performed in positive reflection mode on a Kratos Axima CFR\*.

# MASS SPECTROMETRY



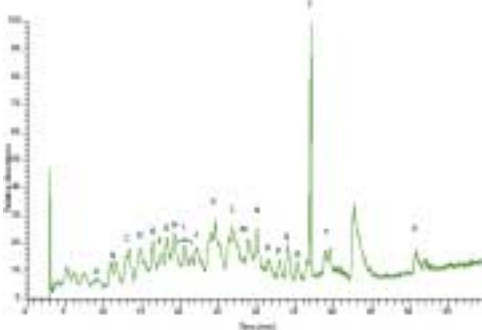
Peak	Tryptic Peptide
A	- 73,103*
B	- 45,64
C	- 2,38
D	- 138*,7,61,77,74,25,68,29
E	- 55,106*,13
F	- 10,41,66,34,35,31,94*
G	- 118*,81*,78
H	- 53
I	- 135*,53,54,18,107*
J	- 941 Da
K	- 63
L	- 125*,48,149*
M	- 42,72,55
N	- 66,184*
O	- 20,87*,86*,66
P	- 124*,46,51,75
Q	- 124*,46,51,75
R	- 25,7,138*,172*
S	- 1247.0 Da
T	- 181*
U	- 31
V	- 93*
W	- 101*,34
X	- 117*
Y	- 126*,96
Z	- 101*,41

**Tryptic Digest Conditions:** A 2 mg/ml solution of Bovine Serum Albumin was reduced with tributylphosphine and alkylated with iodoacetamide (Product Code [PROT-RA](#)). The BSA was digested with Proteomics Grade Trypsin (Product Code [T 6567](#)) at a 1:100 enzyme : substrate ratio in 100 mM ammonium bicarbonate containing 30% acetonitrile. The digest was incubated at 37 °C for 17 hours.



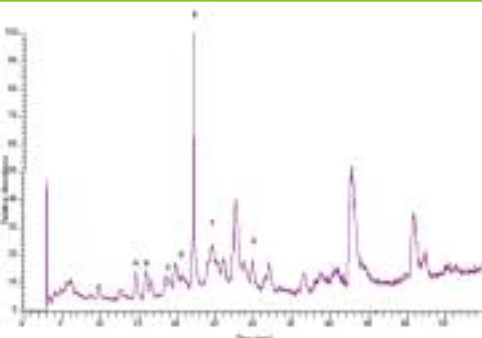
Peak	Arg-C Peptide
A	- 31*
B	- Large Fragment
C	- 19
D	- 10
E	- 18
F	- 37*
G	- 42*
H	- 31*

**Arg-C Digest Conditions:** A 2 mg/ml solution of Bovine Serum Albumin was reduced with tributylphosphine and alkylated with iodoacetamide (Product Code [PROT-RA](#)). The BSA was heated at 100 °C for 10min, cooled on ice then digested with Arg-C (Product Code [P 6056](#)) at a 1:20 enzyme : substrate ratio in 100 mM ammonium bicarbonate. The digest was incubated at 37 °C for 17 hours.



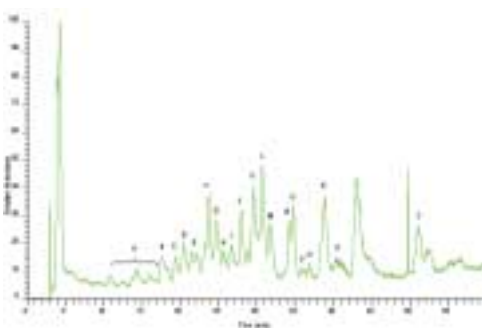
Peak	Glu-C Peptide
A	- 66*
B	- 20,30,48
C	- 91*,45
D	- 844 Da
E	- 51,2,19
F	- 30
G	- 84*
H	- 50,4
I	- 52
J	- 2,19
K	- 16,55,48,9,27,91*
L	- 33,8
M	- 17
N	- 19
O	- 53,25
P	- 15
Q	- 33
R	- 48,97*,43
S	- Large Fragment
T	- 51,97*
U	- 18,54,25

**Glu-C Digest Conditions:** A 2 mg/ml solution of Bovine Serum Albumin was reduced with tributylphosphine and alkylated with iodoacetamide (Product Code [PROT-RA](#)). The BSA was heated at 100 °C for 10min, cooled on ice then digested with Glu-C (Product Code [P 6181](#)) at a 1:20 enzyme : substrate ratio in 100 mM ammonium bicarbonate. The digest was incubated at 37 °C for 17 hours.



Peak	Asp-N Peptide
A	- 70*
B	- 66
C	- 12,19,1
D	- 60*
E	- 31
F	- 137*,21
G	- 36

**Asp-N Digest Conditions:** A 2 mg/ml solution of Bovine Serum Albumin was reduced with tributylphosphine and alkylated with iodoacetamide (Product Code [PROT-RA](#)). The BSA was digested with Asp-N (Product Code [P 3303](#)) at a 1:100 enzyme : substrate ratio in 100 mM ammonium bicarbonate containing 30% acetonitrile. The digest was incubated at 37 °C for 17 hours.



Peak	Tryptic Peptide
A	- 6,18,52,33,46
B	- 50,30,10
C	- 2,20,27
D	- 24,3
E	- 29,2,23,69*14
F	- 55,56
G	- 38,21
H	- 11,38,21,24
I	- 56,5
J	- 5
K	- 30,50,105*,5,41
L	- 30,7,106*
M	- 47,68*
N	- 1477.6 Da
O	- 53
P	- 5,53,79*
Q	- 35
R	- 1651.3 Da
S	- 123*
T	- 23

**Lys-C Digest Conditions:** A 2 mg/ml solution of Bovine Serum Albumin was reduced with tributylphosphine and alkylated with iodoacetamide (Product Code [PROT-RA](#)). The BSA was digested with Lys-C (Product Code [P 3428](#)) at a 1:100 enzyme : substrate ratio in 100 mM ammonium bicarbonate containing 30% acetonitrile. The digest was incubated at 37 °C for 17 hours.

**LC-MS analysis** was carried out using an HP1100 HPLC with a Discovery C-18 (0.21cm x 15cm) 5µ column. A shallow gradient of acetonitrile (0.1% TFA) and water (0.08% TFA) was run. MS data was acquired on an LCQ classic ion trap mass spectrometer in positive ionization mode. Lys-C peptides are numbered in the table. An asterisk indicates a missed cleavage. When no known cleavage could be identified the *m/z* is listed in italics.

# MASS SPECTROMETRY

## Stable Isotope Labeling

With the advance of LC-MS technology, stable isotope labeling has gained increasing attention as a tool for Proteomics. Stable isotope labeled peptides and proteins exhibit near-identical HPLC elution profiles but distinctively different MS spectra, compared to their natural abundance analogs. This unique feature leads to possibility of quantitative study of peptide and proteins, which is vital for proteomics.

Isotec, a member of the Sigma-Aldrich family, provides scientists in proteomics field with a wide range of stable isotope labeled reagents and growth media. With LC-MS, modern NMR techniques, genetic engineering techniques, and a commercial stable isotope supplier, the opportunities for today's biochemists are boundless. Proteomics may be the most dynamic research field in the near future. The only limitation is scientific imagination.

Product Description	Atom%	Product Code	Size
Water- <sup>18</sup> O (97 atom% <sup>18</sup> O)	97% <sup>18</sup> O	<a href="#">32,987-8</a>	250 mg, 1 gm
Suberic acid-2,2,7,7-d <sub>4</sub> Bis(3-Silfo-N-hydroxysuccinimide ester) Disodium salt	98%D	<a href="#">63,234-1</a>	bulk available
2-Bromethanol-1,1,2,2-d <sub>4</sub>	98%D	<a href="#">48,520-9</a>	5 gm
2-Bromoethanol- <sup>13</sup> C <sub>2</sub> , 1,1,2,2-d <sub>4</sub>	99% <sup>13</sup> C, 98%D	<a href="#">60,002-4</a>	bulk available
Acetic Anhydride- <sup>13</sup> C <sub>4</sub>	99% <sup>13</sup> C	<a href="#">48,782-1</a>	250 mg
Acetic Anhydride-1,1'- <sup>13</sup> C <sub>2</sub> ,d <sub>6</sub>	99% <sup>13</sup> C, 99%D	<a href="#">60,745-2</a>	100 mg
Acetic anhydride- <sup>13</sup> C <sub>4</sub> ,d <sub>6</sub>	99% <sup>13</sup> C, 99%D	<a href="#">60,742-8</a>	100 mg
Acetic anhydride-d <sub>6</sub>	99%D	<a href="#">17,564-1</a>	1 gm, 5 gm
Benzoyl-d <sub>5</sub> chloride	98%D	<a href="#">36,604-8</a>	1 gm
Benzoyl-alpha- <sup>13</sup> C Chloride	99% <sup>13</sup> C	<a href="#">27,932-3</a>	1 gm
Bromoacetic Acid- <sup>13</sup> C <sub>2</sub>	99% <sup>13</sup> C	<a href="#">28,383-5</a>	250 mg, 1 gm
Chloroacetyl- <sup>13</sup> C <sub>2</sub> Chloride	99% <sup>13</sup> C	<a href="#">60,408-9</a>	250 mg
Dimethyl- <sup>13</sup> C <sub>2</sub> ,d <sub>6</sub> Sulfate	99% <sup>13</sup> C, 99%D	<a href="#">59,009-6</a>	bulk available
Dimethyl-d <sub>6</sub> Sulfate	99%D	<a href="#">16,452-6</a>	1 gm, 10 gm
Iodoacetamide- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">59,266-8</a>	bulk available
O-Methylisourea- <sup>13</sup> C HCl	99% <sup>13</sup> C	<a href="#">49,317-1</a>	100 mg
O-Methylisourea-1- <sup>13</sup> C, <sup>15</sup> N <sub>2</sub> HCl	99% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,846-7</a>	bulk available
Propionic anhydride-d <sub>10</sub>	98%D	<a href="#">61,569-2</a>	bulk available
Succinic anhydride-1,4- <sup>13</sup> C <sub>2</sub>	99% <sup>13</sup> C	<a href="#">60,390-2</a>	bulk available
Succinic-2,2,3,3-d <sub>4</sub> anhydride	98%D	<a href="#">29,374-1</a>	5 gm
D-Glucose- <sup>13</sup> C <sub>6</sub>	99% <sup>13</sup> C	<a href="#">38,937-4</a>	100 mg, 250 mg, 1 gm
D-Glucose- <sup>13</sup> C <sub>6</sub> , -C-d <sub>7</sub>	99% <sup>13</sup> C, 97-99%D	<a href="#">55,215-1</a>	500 mg, 1 gm, 5 gm
Ammonium- <sup>15</sup> N Chloride	98% <sup>15</sup> N	<a href="#">29,925-1</a>	250 mg, 1 gm, 20 gm
Ammonium- <sup>15</sup> N <sub>2</sub> Sulfate	98% <sup>15</sup> N	<a href="#">29,928-6</a>	250 mg, 1 gm, 10 gm, 20 gm
L-Alanine- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">33,212-7</a>	100 mg, 500 mg
L-Arginine- <sup>15</sup> N <sub>4</sub> HCl	98% <sup>15</sup> N	<a href="#">60,011-3</a>	bulk available
L-Asparagine- <sup>15</sup> N <sub>2</sub> H <sub>2</sub> O	98% <sup>15</sup> N	<a href="#">48,591-8</a>	250 mg
L-Aspartic Acid- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">33,213-5</a>	100 mg, 500 mg
L-Cysteine- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">60,912-9</a>	Inquire
L-Glutamic Acid- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">33,214-3</a>	100 mg, 500 mg
L-Glutamine- <sup>15</sup> N <sub>2</sub>	98% <sup>15</sup> N	<a href="#">49,003-2</a>	250 mg
Glycine- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">29,929-4</a>	250 mg, 1 gm
L-Histidine- <sup>15</sup> N <sub>3</sub>	95% <sup>15</sup> N	<a href="#">57,436-8</a>	Inquire

For larger quantity requests, please contact Isotec at 1-800-448-9760 or (937) 859-1808 or email at [isosales@sial.com](mailto:isosales@sial.com).

# MASS SPECTROMETRY

## Stable Isotope Labeling Continued

Product Description	Atom%	Product Code	Size
L-Isoleucine- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">60,901-3</a>	bulk available
L-Leucine- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">34,096-0</a>	100 mg, 500 mg
L-Lysine- <sup>15</sup> N <sub>2</sub>	98% <sup>15</sup> N	<a href="#">60,902-1</a>	100 mg
L-Methionine- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">60,924-2</a>	1 gm
L-Phenylalanine- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">49,010-5</a>	500 mg
L-Proline- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">60,899-8</a>	500 mg
L-Serine- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">60,900-5</a>	500 mg
L-Threonine- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">60,909-9</a>	250 mg, 500 mg
L-Tryptophan- <sup>15</sup> N <sub>2</sub>	95% <sup>15</sup> N	<a href="#">57,460-0</a>	bulk available
L-Tyrosine- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">33,215-1</a>	50 mg, 250 mg, 500 mg
L-Valine- <sup>15</sup> N	98% <sup>15</sup> N	<a href="#">49,017-2</a>	500 mg
L-Alanine-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">48,988-3</a>	100 mg
L-Arginine-ul- <sup>13</sup> C, <sup>15</sup> N HCl	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,803-3</a>	bulk available
L-Asparagine-ul- <sup>13</sup> C, <sup>15</sup> N H <sub>2</sub> O	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,815-7</a>	100 mg, 500 mg
L-Aspartic Acid-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,783-5</a>	250 mg
L-Glutamic Acid-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,785-1</a>	250 mg, 1 gm
L-Glutamine-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,798-3</a>	100 mg, 500 mg
Glycine-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">48,952-2</a>	500 mg
L-Histidine-ul- <sup>13</sup> C, <sup>15</sup> N	97% <sup>13</sup> C, 95% <sup>15</sup> N	<a href="#">60,800-9</a>	Inquire
L-Isoleucine-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,809-2</a>	bulk available
L-Leucine-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,806-8</a>	100 mg, 250 mg
L-Lysine-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,804-1</a>	bulk available
L-Methionine-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,810-6</a>	bulk available
L-Phenylalanine-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,801-7</a>	250 mg, 500 mg, 1 gm
L-Proline-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,811-4</a>	bulk available
L-Serine-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,813-0</a>	100 mg, 250 mg
L-Threonine-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,777-0</a>	100 mg, 250 mg, 500 mg
L-Tryptophan-ul- <sup>13</sup> C, <sup>15</sup> N	97% <sup>13</sup> C, 95% <sup>15</sup> N	<a href="#">57,459-7</a>	Inquire
L-Tyrosine-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,799-1</a>	bulk available
L-Valine-ul- <sup>13</sup> C, <sup>15</sup> N	98% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,014-8</a>	250 mg, 500 mg, 1 gm
ISOGRO™- <sup>13</sup> C Powder-Growth Medium	99% <sup>13</sup> C	<a href="#">60,686-3</a>	1 gm
ISOGRO™-D Powder-Growth Medium	97-99%D	<a href="#">61,672-9</a>	bulk available
ISOGRO™- <sup>15</sup> N Powder-Growth Medium	98% <sup>15</sup> N	<a href="#">60,687-1</a>	1 gm
ISOGRO™- <sup>13</sup> C, <sup>15</sup> N Powder-Growth Medium	99% <sup>13</sup> C, 98% <sup>15</sup> N	<a href="#">60,683-9</a>	1 gm
ISOGRO™- <sup>15</sup> N,D Powder-Growth Medium	98% <sup>15</sup> N, 97-99%D	<a href="#">60,830-0</a>	1 gm
ISOGRO™- <sup>13</sup> C, <sup>15</sup> N,D Powder-Growth Medium	99% <sup>13</sup> C, 98% <sup>15</sup> N, 97-99%D	<a href="#">60,829-7</a>	1 gm
Deuterium Oxide (sterile and Pyrogen Free)			Inquire
Iodoacetamide- <sup>13</sup> C <sub>2</sub>			Inquire
Iodoacetamide-1- <sup>13</sup> C, <sup>15</sup> N			Inquire
Acetic-d <sub>3</sub> Acid, 2,5-Dioxopyrrolin-1yl Ester			Inquire
Propionic-d <sub>3</sub> acid, 2,5-Dioxopyrrolin-1yl Ester			Inquire
Pentanoic-d <sub>7</sub> acid, 2,5-Dioxopyrrolin-1yl Ester			Inquire
Propionic acid- <sup>13</sup> C <sub>3</sub> , 2,5-Dioxopyrrolin-1yl Ester			Inquire

For larger quantity requests, please contact Isotec at 1-800-448-9760 or (937) 859-1808 or email at [isosales@sial.com](mailto:isosales@sial.com).