Integrated Solutions for Mass Spectrometry

AQUA Peptides for Absolute Quantitation

Custom AQUA Design Guidelines
Multiple Labeled Amino Acids

sigma-aldrich.com
In June 2003, Dr. Steve Gygi and his team presented a strategy for absolute protein quantitation using stable isotope labeled peptides and HPLC-MS.1 This technique, Protein-AQUA™, is based on a common principle: the use of a labeled molecule as an internal standard. Protein-AQUA has often been used in MS analysis of small molecules, but by applying it to protein and peptide analysis, Gygi's team has advanced the abilities of protein researchers to study complex biological samples quantitatively and have provided a valuable new tool for Proteomics.

Figure 1 outlines the process flow of the Protein-AQUA technique. In Step 1 an optimal tryptic peptide is chosen from the protein of interest and synthesized containing one stable isotope labeled amino acid (AQUA Peptide). The native peptide and the synthetic AQUA Peptide share physical properties including size, charge, hydrophobicity, ionic character, and amenability to ionization. When mixed, they elute together chromatographically, migrate together electrophoretically, and ionize with the same intensity. However, they differ in MW from 6 to 10 Daltons depending on which stable isotope amino acid is chosen for incorporation. By mass spectrometry, the native peptide and the synthetic AQUA Peptide are easily distinguishable.

In Step 2, a biological sample containing the protein of interest is extracted and a known amount of synthetic AQUA Peptide is added. The sample is then digested and analyzed by HPLC-MS. In the experiment described by Gerber and Gygi, extracted ion chromatograms were generated for the native peptide and the synthetic AQUA Peptide internal standard. Using peak ratios, the quantity of native peptide was then calculated.

Protein-AQUA has many applications. Gerber and Gygi describe methods to (i) quantify low abundance yeast proteins involved in gene silencing, (ii) quantitatively determine the cell cycle-dependent phosphorylation of Ser1126 of human separase protein, and (iii) identify kinases capable of phosphorylating Ser1501 of separase in an in vitro kinase assay.2

Protein-AQUA is a powerful, enabling technology, the limits of which are only now being explored. For proteomics researchers, it facilitates focused, quantitative studies of not only specific protein expression, but specific amino acid modification as well. How can AQUA work for you?


Sigma recognizes the unique needs of protein researchers and has developed a specialized custom peptide offering to meet the specific requirements of AQUA experimentation. Custom AQUA Peptides are synthesized using fully labeled >98 atom% 13C and 98 atom% 15N enriched amino acids (one labeled amino acid per peptide) and are stringently tested to ensure high purity (HPLC), accurate molecular weight (MALDI-TOF MS), and specific peptide content. Custom AQUA Peptides are available in small (5 x 1 nmol) package sizes to enable convenient sample preparation and provide an appropriate peptide amount.

### Custom AQUA Peptides

- 95% peptide purity by reverse-phase HPLC
- >98 atom% 13C and 98 atom% 15N isotopically labeled amino acid incorporation
- Confirmed molecular weight by MALDI-TOF Mass Spectrometry
- Confirmed peptide content
- Peptides supplied as Trifluoroacetate salt (ammonia acetate salt form also available)
- Phosphorylated amino acids available
- Available in 15 business days

Discover how custom AQUA Peptides can work for you!
Selecting the Best AQUA Peptide

Using the known specificity of trypsin, list all of the tryptic peptides contained in your protein of interest. From these candidate peptides, select the best AQUA peptide for your studies using the guidelines listed below.

- Choose a tryptic peptide which resolves well by HPLC. Avoid peptides that are too hydrophilic or too hydrophobic.
- Choose a peptide which ionizes well.
- Avoid chemically reactive residues (Tryptophan, Methionine, Cysteine), or chemically unstable sequences (Asp-Gly, N-term Gln, N-term Asn).
- Choose a peptide containing an amino acid well-suited to stable isotope labeling, for which the mass difference will be 5 or greater. (Stable isotope labeled amino acid options are listed in the table to the right.)
- Peptide sequence length should be limited to 15 amino acids or less.

Mass Difference between Native Peptide and AQUA Peptide

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Mass Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine-13C6,15N4</td>
<td>10 Daltons</td>
</tr>
<tr>
<td>L-Isoleucine-13C6,15N7</td>
<td>10 Daltons</td>
</tr>
<tr>
<td>L-Leucine-13C6,15N7</td>
<td>8 Daltons</td>
</tr>
<tr>
<td>L-Lysine-13C6,15N2</td>
<td>8 Daltons</td>
</tr>
<tr>
<td>L-Phenylalanine-13C9,15N</td>
<td>10 Daltons</td>
</tr>
<tr>
<td>L-Proline-13C5,15N6</td>
<td>6 Daltons</td>
</tr>
<tr>
<td>L-Valine-13C5,15N6</td>
<td>6 Daltons</td>
</tr>
</tbody>
</table>

Our Unique Expertise

Drawing on the unique strengths of the Sigma-Aldrich family, we are able to provide expertise and excellence to meet Proteomics researchers’ needs for high purity, prequalified, AQUA Peptides.

Sigma-Genosys is recognized as the world’s leading provider of custom peptide and antisera services for the life science research community.

Isotec is the world’s leading commercial manufacturer of enriched stable isotopes and stable isotope labeled compounds.

Sigma-Aldrich is a leader in Life Science and High Technology, producing innovative products for proteomics, recombinant protein expression, high throughput screening, nucleic acid purification, PCR, gene expression, cell signaling, cell culture and chromatography. Sigma-Aldrich R&D scientists are working in our state-of-the-art research and development facility to create the innovations that will help you conduct breakthrough research.

Experience AQUA Peptides for Yourself . . .

1. Identify the best tryptic peptide for your application. We advise performing a tryptic digest on your protein of interest and analyzing by HPLC-MS. This will help determine the best candidate based on peptide resolution and ionization. See “Selecting the Best AQUA Peptide” section above.
2. Determine the sequence of the chosen peptide. Generally, peptides should be fewer than 15 residues long. (If your peptide is longer, prices may increase.)
3. Determine the appropriate stable isotope labeled amino acid to incorporate. Stable isotope labeled amino acids available for AQUA peptides are listed in the table at the top of this page. One residue will be stable isotopically labeled.
4. Place your custom AQUA Peptide order at www.sigma-genosys.com/order.asp or e-mail your order specifications to gorderentry@sial.com. For technical assistance e-mail peptides@sial.com.
5. Your custom AQUA Peptide will be delivered in approximately 15 business days.

Ordering Information

<table>
<thead>
<tr>
<th>Product Description</th>
<th># of peptides x vials x amount</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Custom AQUA Peptide (quantitated by AAA)</td>
<td>1 x 5 x 1 nmol</td>
<td>$500</td>
</tr>
<tr>
<td></td>
<td>10 x 5 x 1 nmol</td>
<td>$4500</td>
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<tr>
<td>Custom AQUA peptide (quantitated with fluorescamine)</td>
<td>1 x 5 x 1 nmol</td>
<td>$400</td>
</tr>
<tr>
<td></td>
<td>10 x 5 x 1 nmol</td>
<td>$3000</td>
</tr>
</tbody>
</table>

When placing your order, please have the following information ready:

- Peptide name
- Peptide sequence
- Stable isotope labeled amino acid of choice
- Required modifications (i.e., post-translational modification)
- Number of packages
- Shipping/Billing address
- Purchase order or credit card number
- Telephone number
- E-mail address
**Pure AQUA Peptide**

Equal amounts of an HPLC purified peptide (GSITEQLL*NAR) and its isotopically labeled analog (GSITEQ**L**QL*NAR) were mixed together. The sample was analyzed by reverse-phase LC-MS using an Agilent 1100 Capillary LC followed by a Finnigan LCQ Classic Ion Trap. Restricted ion current data is presented showing co-elution of the native and isotopically labeled peptides (c) at a one to one ratio.

* indicates the presence of a fully labeled (13C, 15N) amino acid.

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**5 Proteins with AQUA Peptide**

A five protein mixture (containing equal amounts of aldolase, myoglobin, lysozyme, peroxidase, and carbonic anhydrase) was prepared at a final concentration of 1 mg/ml, and digested overnight with Proteomics Grade Trypsin (T 6567) to generate a non-specific background. Equal amounts of an HPLC purified peptide (GSITEQ**L**LNAR) and its isotopically labeled analog (GSITEQ**L**L*NAR) were spiked into the five protein tryptic digestion (a). The sample was analyzed by reverse-phase LC-MS using an Agilent 1100 Capillary LC followed by a Finnigan LCQ Classic Ion Trap (b). Restricted ion current data is presented showing co-elution of the native peptides (c) and isotopically labeled (d) peptides at a one to one ratio.

* indicates the presence of a fully labeled (13C, 15N) amino acid.
Proteomics Grade Trypsin shows excellent proteolytic efficiency, generating more tryptic peptides leading to greater sequence coverage of your protein of interest. Mass spectra are significantly simplified due to the reduced number of interfering autolytic peaks and their ambiguous adducts. Proteomics Grade Trypsin has been extensively purified from porcine pancreas to enable accurate and precise cleavage on the carboxyl side of Arg and Lys residues. The enzyme has been exhaustively processed by reductive methylation to minimize autolysis and chymotryptic activity has been quenched by TPCK (N-p-Tosyl-L-phenylalanine chloromethyl ketone) treatment. Further purification steps including affinity chromatography and lyophilization from dilute acid produce a highly purified, high specific activity trypsin purposely suited for the demanding criteria of proteomics research, and designed to function in either solution or gel based digests. The enzyme is conveniently packaged in 20 µg vials to ensure fresh enzyme is available for each use.

- Highly purified – Eliminates contaminant peaks and non-tryptic fragments from spectra
- Stabilized form retains high specific activity – Complete digestion 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

**Ordering Information**

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
<th>Package Size</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>T 6567</td>
<td>Proteomics Grade Trypsin</td>
<td>5 x 20 µg</td>
<td>$54.60</td>
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