

# Custom Peptides

## Custom Services

For generations of scientists, SIGMA is a company known for integrity and quality, supplying research, biotechnology, and pharmaceutical companies worldwide. Today, in the emerging fields of proteomics and biomarker discovery, we continue this tradition of integrity and quality through Sigma-Genosys, the Quality Provider and Technical Partner for Custom Peptides.

At the foundation of Sigma-Genosys and Sigma-Aldrich is a commitment to integrity, continual process improvement, and strict global quality systems through ISO9001 certification. Through ISO9001, we undergo rigorous internal audits, supplier qualifications, corrective and preventative actions, as well as training programs – all designed to offer the highest quality products to our customers.

### Sequence Design and Analysis

Sigma-Genosys offers design analysis of peptide sequences for all quotation and order requests. Understanding the chemistry behind the synthesis, as well as identifying potential issues that might be encountered during the manufacturing process, allows the optimal synthesis and purification strategies to be implemented for the desired peptide sequence.

Design experts are available to assist in the technical analysis and design for peptides and antigens.

### Peptide Design & Analysis Tool ([sigma.com/peptides](http://sigma.com/peptides))

This web-based tool provides calculations for the desired peptide sequence. This analysis tool will identify any potential chemistry issues and make suggestions for minimizing the issues that might be encountered during the manufacturing process.

### Peptide Synthesis Design Elements

- Aggregation profiling: predicts the efficiency of the peptide bond formation between amino acids as the peptide chain is being synthetically formed
- Solubility prediction: hydrophobicity values at pH 2 and pH 6.8 are presented for each peptide sequence
- Peptide chemistry: highlights potential chemistry issues that may impact the customer application. Suggestions are presented for altering the peptide sequence to reduce or eliminate the predicted chemistry issues

## Custom Peptide Services

Synthetic custom peptides are an important tool in drug discovery and proteomic research. Sigma-Genosys provides a comprehensive service portfolio to assist customers in applications requiring custom peptides, peptide libraries, and antibodies.

### Custom Peptide Synthesis Specifications

- Amounts: 5 mg to 10 g
- Sequence length: up to 50 amino acids
- Purity levels: Immunological grade (>50%), >70%, >80%, >95%
- QC: MALDI-TOF mass spectrometry and reverse-phase HPLC provided for all peptides (Amino Acid Analysis is available upon request)

### Modification Offering

Sigma-Genosys routinely provides modified peptides to the research community, with a dedicated team focused on producing specialty peptides. The most commonly requested modifications include:

- Biotin
- Fluorescein
- FRET peptides
- Phosphorylation
- Non-standard residues
- D-amino acids
- Disulfide bridge formation (cyclization)
- Stable isotope incorporation

We continually add to our modification offering. Contact [peptides@sial.com](mailto:peptides@sial.com) with specific modification requirements.

# Custom Peptides

## Application Recommendations

Purity Level	Application
Immunological Grade & >70% Purity	<ul style="list-style-type: none"> <li>■ Antigen for antibody production</li> <li>■ ELISA standard for measuring titers of antibodies in antisera</li> <li>■ Competitive elution chromatography</li> <li>■ Peptide array production</li> </ul>
>80% Purity	<ul style="list-style-type: none"> <li>■ Non-quantitative enzyme-substrate studies</li> <li>■ Phosphorylation assays</li> <li>■ Non-quantitative peptide blocking studies</li> <li>■ Coupling to resin for affinity purification</li> <li>■ Coating of tissue culture plates for cell attachment</li> <li>■ Protein electrophoresis applications</li> </ul>
>95% Purity	<ul style="list-style-type: none"> <li>■ Standards for quantitative ELISA and RIA protocols</li> <li>■ Quantitative receptor-ligand interaction studies</li> <li>■ <i>In vitro</i> bioassays</li> <li>■ <i>In vivo</i> studies</li> <li>■ Quantitative enzyme studies</li> <li>■ NMR studies</li> <li>■ Mass spectrometry</li> <li>■ Quantitative assays</li> </ul>

## Peptide Synthesis

Fmoc chemistry and a solid support resin. Synthesis is conducted from the C-terminus of the sequence to the N-terminus of the sequence with repeated deprotection, activation and coupling cycles.

## Peptide Purification

Following synthesis, peptide materials not meeting the specified purity requirement are purified by mass-directed HPLC purification systems based upon reverse phase chromatography technology. Mass-directed purification systems are employed to obtain a better quality of peptide material, while significantly increasing purification throughput. In addition, analytical capabilities have been augmented by the introduction of MS/MS detection to ensure the highest quality and peptide integrity.

## Quality Control

Every peptide synthesized by Sigma-Genosys is controlled for quality using a combination of MALDI-TOF mass spectrometry and reverse-phase HPLC chromatography. MALDI-TOF MS is essential in confirming the peptide composition based on the molecular mass of the peptide. Reverse-phase HPLC chromatography verifies the purity level of the product. Amino acid analysis (AAA) can be performed to further verify the product composition and determine the net peptide content present in the sample.

## Dispatch Guidelines

Manufacturing time is dependent on the complexity of the peptide sequence. Estimated dispatch times are listed in the table below. In the event there are unexpected delays in the manufacturing process, our customer service representative will notify the customer with adjusted delivery estimates.

## Estimated dispatch guidelines (in business days) based on peptide amount, length, and purity required

	Immunological Grade			>70% Purity			>80% Purity			>95% Purity		
	≤20 aa	>20 aa	>30 aa	≤20 aa	>20 aa	>30 aa	≤20 aa	>20 aa	>30 aa	≤20 aa	>20 aa	>30 aa
5 mg-25 mg	10	20	25	10	20	25	15	20	25	20	25	30
50 mg-100 mg	15	30	35	20	30	35	20	30	35	30	35	40

# Custom AQUA Peptides™

## Custom AQUA Peptides

Protein AQUA is a powerful tool, enabling technology that facilitates focused, quantitative studies of not only specific protein expression, but specific amino acid modification as well. As proteomic research evolves from biomarker discovery to validation, choose the best strategy to accurately quantitate the specific biomarkers of interest.



## Sigma Custom AQUA Peptides

To meet the specific requirements of AQUA experimentation, Sigma has developed a specialized custom peptide offering. Custom AQUA peptides are synthesized using fully labeled 98 atom %  $^{13}\text{C}$  and 98 atom %  $^{15}\text{N}$  enriched amino acids (one labeled amino acid per peptide), and are stringently tested to ensure high purity (HPLC), accurate molecular mass (ESI-MS), and specific peptide content (AAA).

Custom AQUA peptides are available in small (5 × 1 nmol) package sizes to enable convenient sample preparation, and provide an appropriate peptide quantity.

- 95% peptide purity by reverse-phase HPLC
- 98 atom %  $^{13}\text{C}$  and 98 atom %  $^{15}\text{N}$  isotopically labeled amino acid incorporation
- Confirmed molecular mass by ESI Mass Spectrometry
- Confirmed peptide content by AAA
- Phosphorylated amino acids available
- Available in 25 business days

## Selecting the Best AQUA Peptide

Selecting an optimal AQUA peptide is key to your analytical success. Because your time and resources are valuable, we suggest a careful evaluation of candidate peptides. The following guidelines provide a good starting point.

Using the known specificity of trypsin and the sequence of your protein of interest, generate a list of all of its tryptic peptides. Using the guidelines below, eliminate unsuitable peptides and select peptides with optimal characteristics for AQUA experimentation.

- Choose a peptide that is unique to your protein of interest
- Choose a tryptic peptide which resolves well by HPLC, avoid peptides that are too hydrophilic or too hydrophobic
- Choose a peptide which ionizes well in your MS system
- Avoid chemically reactive residues such as Tryptophan, Methionine, Cysteine, or chemically unstable sequences such as Asp-Gly, N-term Gln, and N-term Asn
- Choose an amino acid from the available list to be isotopically labeled
- Peptide sequence length should be limited to 15 amino acids or less

Our design experts are available to assist in determining the optimal AQUA peptide. Email your protein sequence to [peptides@sial.com](mailto:peptides@sial.com) for assistance with AQUA design.

## Available AQUA Amino Acids

Stable Isotope Labeled Amino Acid (Fully Labeled, $^{13}\text{C}$ , $^{15}\text{N}$ >98 atom %)	Mass Difference between Native Peptide and AQUA Peptide
L-Arginine- $^{13}\text{C}_6$ , $^{15}\text{N}_4$	10 Daltons
L-Isoleucine- $^{13}\text{C}_6$ , $^{15}\text{N}$	7 Daltons
L-Leucine- $^{13}\text{C}_6$ , $^{15}\text{N}$	7 Daltons
L-Lysine- $^{13}\text{C}_6$ , $^{15}\text{N}_2$	8 Daltons
L-Phenylalanine- $^{13}\text{C}_9$ , $^{15}\text{N}$	10 Daltons
L-Proline- $^{13}\text{C}_5$ , $^{15}\text{N}$	6 Daltons
L-Valine- $^{13}\text{C}_5$ , $^{15}\text{N}$	6 Daltons

# Custom Peptides



## PEPscreen: Custom Peptide Libraries

To accommodate the flexibility requirements of custom peptide libraries, Sigma-Genosys has developed a proprietary, state-of-the-art, robotic platform to synthesize different length peptides in high quality and in a parallel, high throughput manner. The PEPscreen service provides researchers the ability to order large numbers of affordable custom peptide sets delivered in as little as 7 business days.

### PEPscreen Specifications

- Peptide length: 6 to 20 amino acids
- Guaranteed gross weight: 0.5 mg to 2 mg
- QC: MALDI-TOF mass spectrometry
- Peptide form: dried film at the bottom of individual tubes
- Packaging: 96-well tube rack format (2-D barcode)
- Minimum order size: 24 peptides

### Modifications Available

- N-termini: free amine or acetylated
- C-termini: free acid or amidated
- Non-standard amino acids: any commercially available non-standard amino acid
- Chemical modifications: cyclization, phosphorylation, biotinylation, PEGylation, acylation, etc.
- Dye labeling: Fluorescein, Dansyl, Dabcyl, Dabsyl, TAMRA, Lissamine, etc.
- Random library: any mixture of commercially available amino acids

### Peptide Library Design Tool

Sigma-Genosys has created an online design tool for creating sequences for peptide libraries.

- Ability to set custom sequence parameters (peptide length and amino acid offset)
- Format the sequence output
- Export the custom peptide library sequences to other programs (Microsoft Word, Excel, etc.)
- Calculated hydrophathy for each peptide sequence
- Color-coded amino acids for visual reference of peptide sequence composition

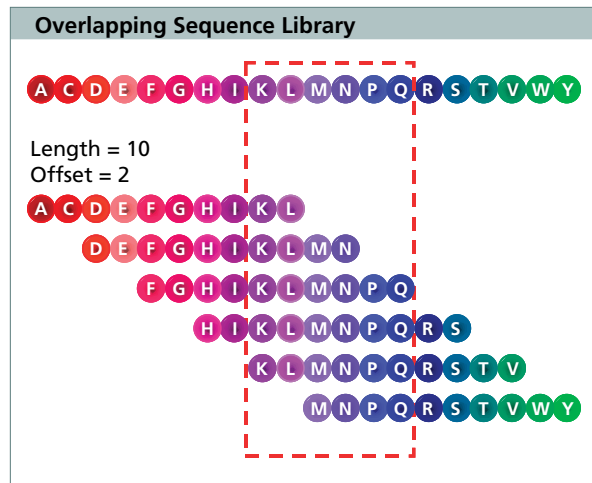


To learn more and utilize this technical resource design tool, visit: [sigma.com/pepscreen](http://sigma.com/pepscreen).

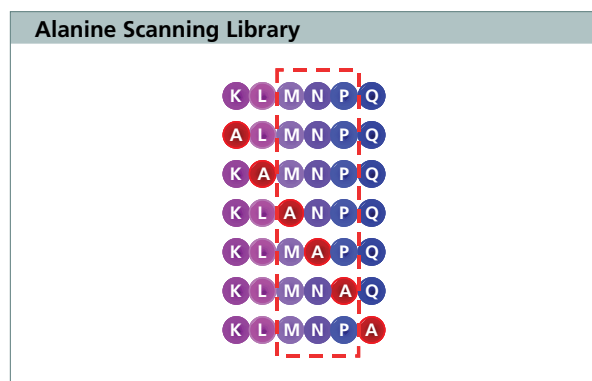


## Types of Peptide Libraries Available

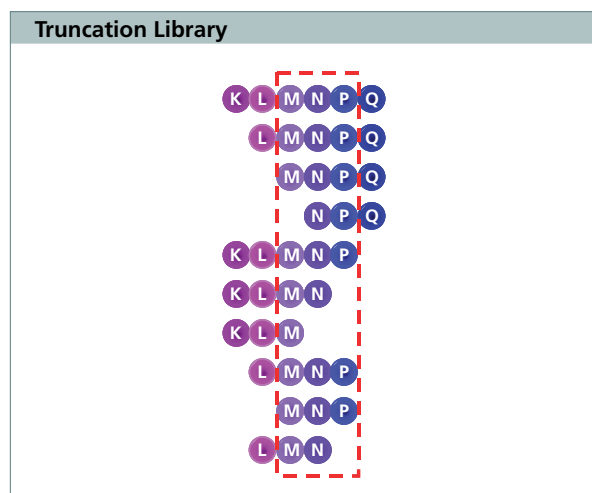
- Overlapping sequence library:** synthesis of overlapping peptides to span the entire protein sequence. The example shows an overlapping peptide library consisting of 15 aa with an offset of 5 aa (10 aa overlap)



- Alanine scanning library:** synthesis of peptides in which alanine is systematically substituted for each of the amino acid positions in the previously identified epitope

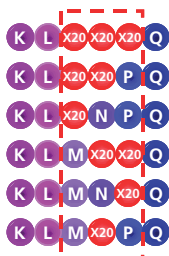


- Truncation library:** series of peptide sequences representing the systematic truncations of the flanking residues to determine the minimum length required for optimum peptide activity



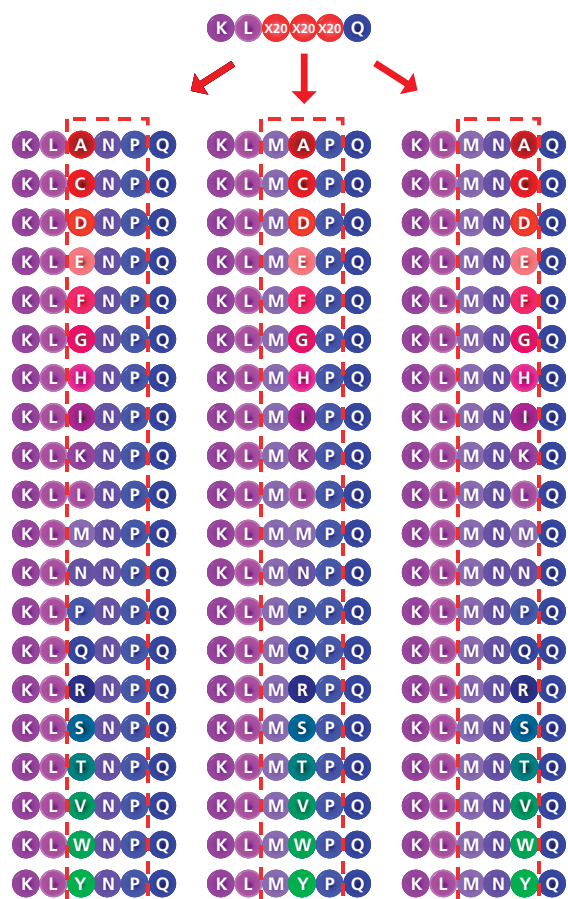
# Custom Peptides

## Random Library



- **Random library:** selected residues in the peptide sequence (wobble sequence) are simultaneously substituted with a mixture of all 20 amino acids, or a mixture of pre-determined amino acids

## Positional Scanning Library



- **Positional scanning library:** selected positions in a peptide sequence are each systematically replaced with a different amino acid for determining the preferred amino acid residues at these positions, as manifested by corresponding increases in activity



# Custom Antisera

## Custom Antisera Services

Antibodies to peptides have become an essential tool in life science research, with applications including gene product detection and identification, protein processing studies, diagnostic tests, protein localization, active site determination, protein homology studies, and protein purification.

### Antigen Design

Sigma-Genosys routinely assists researchers with antigen design. The antigen design elements include:

- Protein surface exposure and flexibility: the proven algorithms of Hopp and Woods hydrophilicity scale and Kyte and Doolittle hydrophobicity scales are used for identifying predicted antigenic regions of the protein
- Sequence homology: comparing the known sequence of interest to others using the BLAST algorithm
- Conjugation attachment chemistry: attachment chemistry of the peptide to the carrier protein (antigen development). The attachment chemistry is important for presenting the protein fragment (peptide) to the immune system in a similar orientation as if in the native protein
- Peptide chemistry: review of the desired antigenic peptide for synthesis issues that may arise during the manufacturing process of the antigen

### Antigen Development

Peptides are synthesized using solid phase Fmoc chemistry. Typically 10 mg are produced, in which 3 mg are conjugated to the carrier protein. The conjugated peptide (antigen) is used for animal immunizations. The balance of the peptide (unconjugated) is supplied to the customer.

### Host Animals

When attempting to generate an antibody, immunizations should be performed in animals that are as far in evolutionary distance from the antigen source as possible.

Host Animal	Species	Typical Dosage	Total Antigen Amount Required	Sera Volume (per bleed)
Rabbit	New Zealand White	0.2 mg (first dosage; 0.1 mg (remaining dosages)	0.7 mg	25 ml
Chicken	Rhode Island Red	0.1 mg (first dosage; 0.05 mg (remaining dosages)	0.35 mg	1-2 ml (15 ml per yolk)
Goat	Nubian	0.4 mg (first dosage; 0.2 mg (remaining dosages)	1.5 mg	200 ml
Sheep	Suffolk	0.4 mg (first dosage; 0.2 mg (remaining dosages)	1.5 mg	200 ml

### Immunization and Sera Collection

The antigen is injected subcutaneously into two host animals. The initial immunization is emulsified using Complete Freund's Adjuvant and all subsequent immunizations emulsified using Incomplete Freund's Adjuvant prior to injection. A pre-immune bleed is drawn from each animal and is supplied as a control. Four bleeds (including the pre-immune bleed) are drawn from the animals and shipped to the customer on demand. All sera are supplied unpurified and contain a number of different isotypes (IgG, IgM, and IgA). Sera purification is available upon request. The standard immunization and bleed protocol is listed below.

Day 0	Pre-Immune Bleed, Antigen Injection #1
Day 14	Antigen Injection #2
Day 28	Antigen Injection #3
Day 42	Antigen Injection #4
Day 49	Production Bleed #1, ELISA
Day 56	Antigen Injection #5
Day 63	Production Bleed #2
Day 70	Antigen Injection #6
Day 77	Production Bleed #3

### Package Options

The package options are designed to offer different components to best fit customer needs.

**The Partial Package:** using researcher-supplied proteins, peptides, or antigens for antisera production. Our partial service follows the standard immunization protocol as shown above.

**The Complete Package:** includes comprehensive antigen design, antigen development (peptide synthesis and conjugation to the carrier protein) as well as the standard immunization protocol.

**Multiple-Peptide (Co-Immunization) Package:** strategy in which multiple peptide sequences, representing different epitopes of the same protein, are separately conjugated to a protein carrier, and each conjugate is then mixed together and formulated for immunization into the same pair of animals. Since each carrier protein molecule is conjugated to only one type of peptide, this may enable distinct recognition and T cell responses to each peptide antigen. This service includes the antigen design, antigen development (synthesis of multiple peptides and conjugation of each peptide to a carrier protein) as well as the standard immunization protocol.

# Custom Antisera

**Monospecific/Characterized Package:** includes all of the services outlined for The Complete Package with the addition of animal exsanguination (terminal bleed), peptide affinity purification, and Western blot analysis. The Monospecific/Characterized Package can replace the need for monoclonal antibodies in many research applications. This polyclonal package provides researchers with the specificity of monospecific polyclonal antibodies with a quicker time-frame, when compared to monoclonal antibody production.

**Phosphospecific Package:** strategy in which a phosphorylated peptide acts as the antigen for eliciting the immune response. This package includes the antigen development of a phosphorylated peptide (>95% purity, 10 mg), the non-phosphorylated companion peptide that will be used during the affinity purification process, standard immunization protocol, ELISA of bleed #1 using the phosphorylated peptide and the non-phosphorylated peptide, and affinity purification of the sera. The affinity purification process includes depletion of the non-phosphospecific antibody and the purification of the phosphospecific antibody (double peptide affinity purification using the non-phosphorylated peptide and the phosphorylated peptide).

## Sera Purification

**Sigma-Genosys offers 3 methods of sera purification.**

1. Ammonium sulfate precipitation is a fairly crude non-specific purification, which removes the majority of plasma proteins and leaves the immunoglobulin fraction.
2. Protein A or Protein G purification yields an enriched IgG preparation.
3. Peptide affinity purification allows for the purification of highly specific antibodies from the crude sera. Unlike Protein A or G, the non-specific Ig fraction is not retained. Sigma-Genosys purifies 20 ml of sera from the last bleed from the chosen animal. The peptide affinity service includes construction of the column, purification of 20 ml of sera, and an ELISA on the crude and purified sera. The column and purification protocol are supplied, should you elect to purify additional amounts of sera.

## Custom Antisera Guarantee

Sigma-Genosys guarantees the production of an antiserum that will recognize the desired peptide by ELISA. However, we do not make claims as to the ability of antisera to recognize the target protein.

Should there be a failure to produce an antiserum that recognizes the desired peptide by ELISA, Sigma-Genosys will either credit the antisera portion of the service or begin the immunization schedule using a new antigen. Sigma-Genosys requires notification as soon as a problem is detected and within 30 days of receiving the sera from the last production bleed.

Sigma-Genosys continually adds technical content to the website ([sigma.com/peptides](http://sigma.com/peptides)).

Use this site as a reference for:

- Design tools
- Troubleshooting Guides
- Protocols
- Frequently Asked Questions (FAQ)
- Storage & Handling Guidelines
- Solubilization Suggestions
- And much more...

