

CUSTOM PEPTIDES AND ANTISERA

Custom Peptide Synthesis

As one of the world's largest producers of synthetic peptides, Sigma-Genosys has the manufacturing facilities, the technical expertise and the proprietary instrumentation needed to fulfill your order in a professional and timely manner. Over the years we have established ourselves as a quality supplier and technical partner with customers.

Quality and Technical Analysis:

Our company policy is based around quality. We are an ISO-9001:2000 registered company (Certificate number QSR-773). All sequences are analyzed by software at the time of quotation or order. Sequences are analyzed for hydrophobicity at pH2 and pH6.8, aggregation potential and other known chemistry issues that may arise due to the peptide's amino acid composition. The customer is notified of any potential issues at the time of quotation or order so that sequence modifications can be made prior to synthesis, if possible. The Sigma-Genosys website provides the technical analysis output for peptide sequences.

If specifications of the order are not achieved, the customer is immediately notified of the issues. At this time, the customer will be consulted and given technical advice on redesigning the sequence, or exploring alternative chemistry. The customer may be offered the material that is achieved at a discounted rate.

Basic Peptide Synthesis Overview:

Our patented segmented continuous-flow synthesis (*Abacus* technology) allows us to synthesize peptides quickly and with an exceptional degree of purity. Because our patented synthesizers are developed in-house we have engineering and maintenance support available 24 hours each day, minimizing manufacturing delays.

Peptide synthesis is performed by using 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.

Standard Product Offering:

Sigma-Genosys offers peptide amounts from 2 mg to 1 gram. We synthesize peptides from 5 to 120 amino acids in length. Purity levels include Immunological grade (>50% purity), >70%, >80%, and >95%. The table below shows recommended purity levels based on common applications.

Modification Offering:

Sigma-Genosys routinely provides modified peptides to the research community. We continually add to our modification offering. Contact us with your specific modification requirements.

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

The most commonly requested modifications include:

- Biotin (N-terminal and C-terminal)
- Fluorescein (N-terminal and C-terminal)
- Cyclization
- Phosphorylated residues
- D-amino acids
- Lissamine Rhodamine
- Black Hole Quencher™ Dyes
- Non-standard residues
- FRET peptides

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Applications using PEPscreen™ product include but are not limited to:

- Epitope Mapping
- Protein-Protein Interactions
- Alanine Scan
- Receptor-Ligand Interactions

Applications using SPOTs technology:

- Epitope mapping
- Protein-protein and receptor-ligand interaction
- Phosphorylation studies
- Metal binding studies
- Nucleic acid binding studies
- Signal-transduction studies

PEPscreen™: Custom Peptides for Screening Applications

Introducing affordable, quality-assured peptides in a 96-well format. Sigma-Genosys has developed a proprietary peptide synthesis platform using state of the art technology. This technology provides a solution to enhance your peptide screening applications. As a result, large numbers of peptides are delivered quickly and affordably, offering great value for your money.

MALDI-TOF mass spec is run on 100% of your samples. We invest in Quality Control, so you do not waste time validating results.

PEPscreen™ offering:

- MALDI-TOF Mass Spectrometry on 100% of peptides
- Yield: ~0.5-2 mg
- Purity: ~70% (based on 15mers)
- Length: 8-20mers
- N-terminal modifications and non-standard residues available
- Peptides supplied lyophilized and in 96-well format (2-D bar coded tubes)
- Delivery: ~7 working days per run
- Minimum order size is 48 peptides

SPOTs Protein Mapping Technology

SPOTs peptide synthesis technology enables easy identification of the functional domains of proteins. By conducting functional assays on peptide libraries covalently attached to derivatized cellulose membranes, the amino acid sequence of protein antigenic sites can be determined.

Peptide syntheses typically yield between 5-10 nmoles of peptide per spot (6-12 µg for an average 10mer). The peptides are covalently attached to the membrane, which allows the membrane to be stripped and regenerated.

Custom SPOTs Service

Sigma-Genosys will synthesize your peptides, according to your requirements, onto a derivatized cellulose membrane. The membrane is sent to you with the peptides attached and ready for analysis.

Custom Peptide Antisera Production

Sigma-Genosys will assist you throughout the entire antisera production process. We begin with antigen design assistance, if needed. Once the target peptide sequence has been selected we synthesize and conjugate the peptide to a carrier protein. The antigen is injected into two host animals and after a series of immunizations; blood is drawn from the animals.

Peptide Design and Synthesis:

Sigma-Genosys routinely assists customers with the antigen design. Antigen design is the most critical aspect of antibody production. We use sophisticated software to determine the best regions on the protein of interest based on antigenicity (hydrophilicity), flexibility, secondary structure and aggregation potential. The complexity of manufacturing the peptide is also considered.

Once the sequence has been designed, we'll synthesize the peptide using our proprietary *Abacus* synthesis technology and Fmoc chemistry. All peptides are analyzed for composition using mass spectrometry and for purity using reversed phase HPLC. Typically 10-15 mg of peptide are synthesized, of which 2-3 mg are conjugated to the carrier protein of choice.

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Custom Peptide Antisera Production continued

Conjugation:

Sigma-Genosys offers a wide variety of carrier proteins as well as different conjugation chemistries. Keyhole limpet hemocyanin (KLH) is the common carrier protein choice for most protocols. KLH is thought to be more immunogenic than bovine serum albumin (BSA) because it is a non-mammalian protein.

Conjugation chemistry is a critical aspect in antigen design, however it is often overlooked by the researcher. Conjugation chemistry refers to the method of attaching the peptide to the carrier protein. It is important to conjugate the peptide to the carrier protein in a manner that is most similar to how the peptide segment occurs in the native protein of interest. This consideration will increase your odds of obtaining an antibody that recognizes the native protein of interest. Refer to our website for conjugation chemistry strategies.

Immunization and Sera Collection:

The conjugation peptide is injected subcutaneously into two host animal. 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 as they are drawn. The customer also receives the remaining unconjugated peptide sample. All sera are supplied unpurified and contains a number of different isotypes (IgG, IgM, and IgA). Purification can be requested (see Optional Services section).

Optional Services

The Partial Service:

Sigma-Genosys routinely accepts antisera projects using researcher-supplied proteins, peptides or antigens. Our partial service follows the same 77-day immunization protocol as listed above. *Sigma-Genosys cannot guarantee an immune response to customer-supplied antigens.*

Continuation Service:

The immunization / bleed protocol may be continued beyond the 77-day schedule on a monthly basis for one or both animals. Additionally, customers can schedule an exsanguination at the end of the standard protocol to collect all sera.

Host Animals:

Two New Zealand White rabbits are the standard host animals. Additional host animals include chickens, goats and sheep. The table below lists the sera yields per animal.

Sera Purification:

Sigma-Genosys offers 3 methods of sera purification.

- Ammonium Sulfate Precipitation is a fairly crude non-specific purification, which removes the majority of plasma proteins and leaves the immunoglobulin fraction.
- Protein A or Protein G Purification yields an enriched IgG preparation.
- Peptide Affinity Purification allows for the purification of highly specific antibodies from the crude sera.

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 within 30 days of receiving the sera from the last production bleed.

Contact Sigma-Genosys at 1-800-234-5362 or www.sigma-genosys.com for more information.

Standard Immunization & Bleed Protocol

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

Animal Type	Sera per animal per bleed	Total Sera Provided
Rabbit	~20 ml	~150-175 ml
Chicken	~1-2 ml	~12 ml (12 egg yolks)
Sheep	~200 ml	~1400 ml
Goats	~200 ml	~1400 ml