PepSets™ and their Applications

PepSets peptide libraries are the rapid and cost effective solution for an immense range of bioactivity screening purposes

Topics in this article:

Introduction to PepSets

Peptide libraries are a powerful tool in biological research for screening large numbers of peptides in the search for the few, critical bioactive peptides.

Mimotopes PepSets are custom-synthesized peptide libraries supplied unpurified for fast, efficient screening work. These sets of chemically synthesized individual peptides are made on a small scale using Mimotopes' unique proprietary parallel array synthesis platform. PepSets comprising hundreds, or even thousands of peptides, are rapidly synthesized and shipped within a few weeks of ordering.

Initial screening can be done with peptides of sufficient purity for the purpose, rather than with highly purified peptides which are relatively expensive and slow to manufacture. In accord with this idea, Mimotopes' peptide libraries are made on a small scale, subsequently progressing to larger quantities, or purifying the peptides, when it is known which peptides are going to be useful in detailed studies. This approach represents a huge time and cost saving for the user.

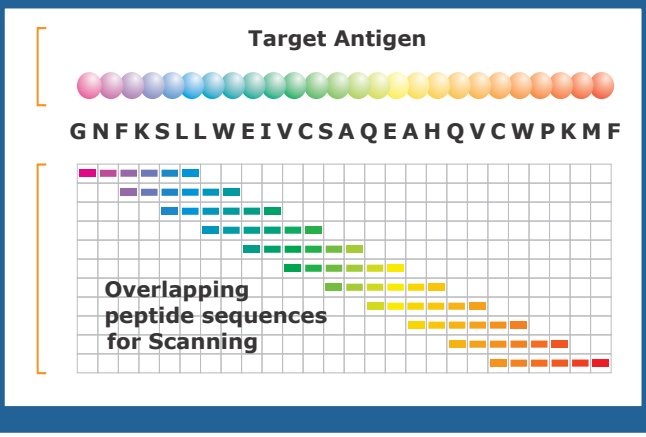
For users who require libraries of purified peptides, Mimotopes also offers a conventional purified custom peptide service.

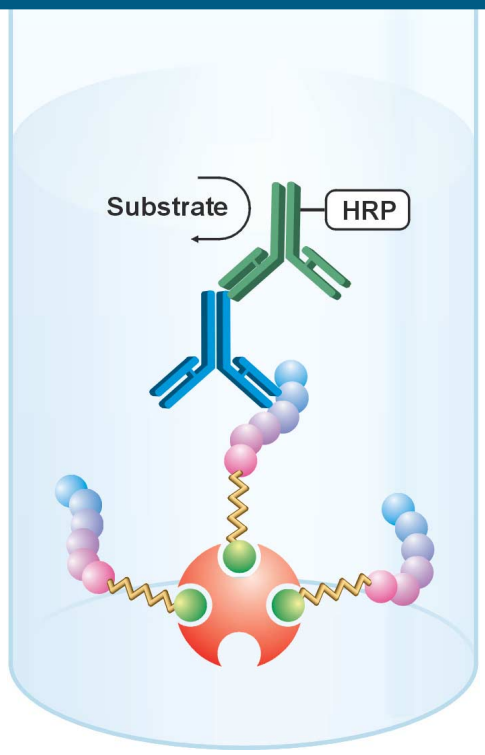
How are PepSets Used

Peptide Libraries are used for diverse purposes, from peptide epitope mapping and structure-activity studies, to the search for peptide "drugs".

PepSets are ideal for T cell epitope searching, because T cell epitopes are by nature short linear peptides from the primary protein sequence. They are also appropriate for scanning the primary sequence of proteins for linear, or "continuous", antibody-defined epitopes.

Where a bioactive sequence is already known, PepSets provide the ideal primary set of analogs for screening in structure-activity relationship (SAR) studies, also known as "Analoging". To discover novel bioactive peptides, a PepSet consisting of many altered sequences, with natural or unnatural amino acid replacements, is synthesized and tested. This can result in the definition of highly promising candidate peptide therapeutics, or in the exclusion of classes of inactive peptide analogs from further study.





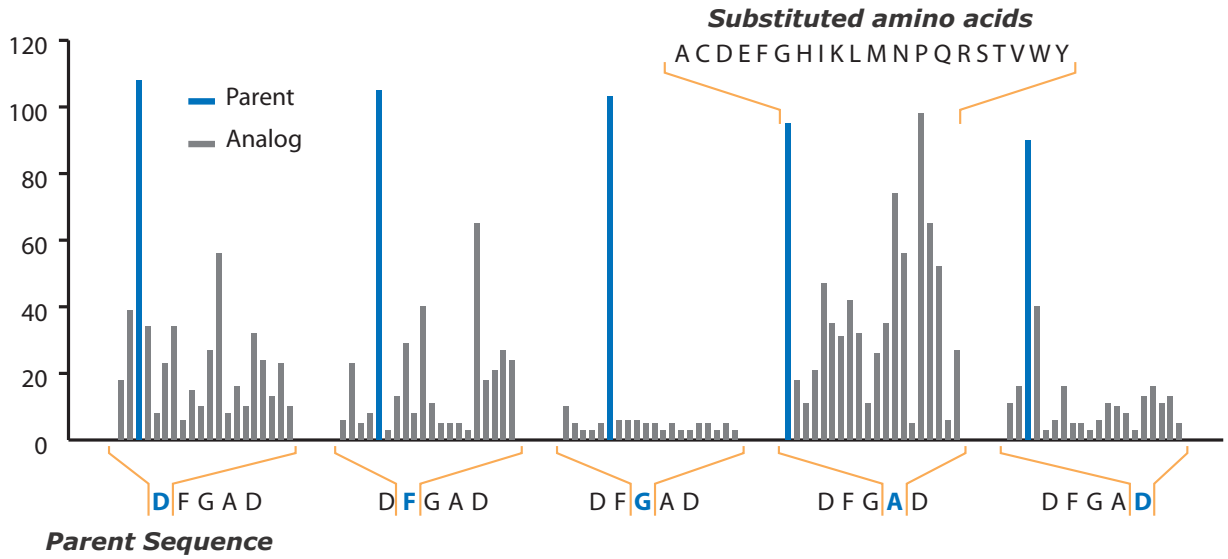
Biotinylated PepSets
Addition of a biotin group to a peptide is a convenient way to detect or immobilize the peptides in libraries.

Benefits of PepSets

The cost of manufacturing and hence purhcasing PepSets is kept down thanks in part to Mimotopes' unique proprietary solid-phase technology and also to their simultaneous synthesis in an unpurified, 96-well array format which is ideal for screening purposes. This also allows many more peptides to be made and tested than would be possible if every peptide was individually characterized before bioassay. Peptides found to be bioactive can then be obtained in purified form for confirmatory experiments and for larger-scale work.

Owing to the economies of scale of parallel synthesis, the average cost of PepSets peptides decreases as the size of the set increases. In addition, because of its unique Synthesis technology, Mimotopes can choose the appropriate scale of synthesis for your PepSets. You therefore pay only for what you need and not for excess mass due to larger synthesis scale technologies.

- **Novel designs**
  Mimotopes has the ability to fine tune the overall design of each synthesis to suit the particular application. For example, peptides for an antibody-binding experiment may be supplied as powders ready for redissolving; as coated plates ready for an ELISA; or as re-usable, permanently bound peptides on a water-wettable polymer surface. Another example is peptide libraries incorporating unnatural or unusual amino acids, tags, or linkers.



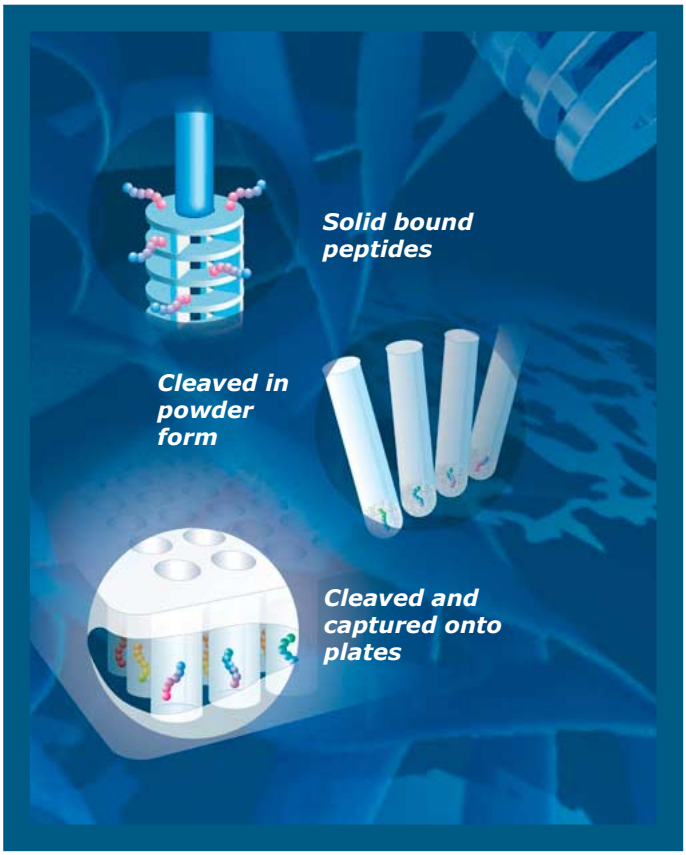
Application of a PepSet to Analoging
Each amino acid of sperm whale myoglobin pentapeptide epitope 122DFGAD126 is replaced in turn with one of the 19 alternative amino acids to determine replaceability (Structure-Activity Relationships) in an antibody binding assay.

- **Quality Built In**
  To assure utmost quality, Mimotopes always synthesizes Control Peptides with every set. Two peptides are made per plate (of up to 94 peptides) and, for Cleaved PepSets, are fully analyzed by HPLC, MS and AAA as a test of the fidelity of synthesis. Only after these Control peptides have passed scrutiny for correct identity, purity and yield, is the set of peptides released for shipping. Working from a verified database during synthesis, computerized equipment specifies the addition of each amino acid, ensuring correct assembly of the sequences. Cleaved PepSets are shipped as dry powders in convenient 96-tube racks containing up to 94 client peptide tubes per rack, less than the original 96 peptides because of the removal of the two Control Peptides.

- **Fast Synthesis**
  PepSets are fast to make, because the peptides are made using a unique parallel synthesis technology. Typically, PepSets of 100 to 1,000 peptides can be supplied within 4 weeks. Larger sets, e.g. 10,000 peptides, will take longer. All peptides can be shipped at the same time, and thus can be screened simultaneously to obtain full data sets under identical conditions.

- **Direct Answers to Vital Questions**
  Mimotopes peptide libraries are frequently the fastest, most economical and most direct way to answer vital questions about a vaccine, diagnostic, or peptide-related drug development project. For example, the peptide recognized by a cytotoxic T cell is readily identified using a set of overlapping peptides spanning the sequence of the target protein. Once the answers from this direct screening methodology are in hand, decisions about the direction of the project can be made without the risk of running a long expensive program with an uncertain outcome.

- **User-friendly product presentation**
  This includes helpful technical guides, a Decapping Tool and spare tube caps for Cleaved PepSets, plus expert technical support for the inexperienced user.



Features and Formats

PepSets are available in numerous formats to suit a wide range of applications:

- **Cleaved or noncleavable format**
- **High or low synthesis scale**
- **Huge variety of overall designs for the peptide library**

Specific PepSets formats have been tailored for common applications in immunology and drug discovery. The table below highlights the most popular formats and their areas of use.

PepSets for specific applications

Description	Application
Truncated PepSets	Used for T-cell epitope mapping
Cyclic PepSets	Used in peptide applications requiring conformational restraint
Biotinylated PepSets	Binds to avadin to rapidly scan protein sequences for bioactive sites
Phosphorylated PepSets	Used for detecting signal transduction protein binding
FRET PepSets	For investigating protein interactions such as those involving proteases
Cell Penetrating PepSets	Investigating enzyme inhibitors for use as peptide hormones and drugs
Multiple Antibody PepSets	For systematic monoclonal antipeptide antibody discovery

Applications in Biological Research

Some of the most frequently used applications are listed below.

- **Immunology** [1,2]
  The most common use of PepSets in immunology is for B- and T-cell Epitope Mapping. Table 1 is an overview of the design guidelines for epitope mapping.

- **Pharmacology/Physiology** [3]
  The most common use of PepSets in pharmacology/physiology is for locating or analoging of pharmacologically active peptides such as neurotransmitters, hormones or chemokines. Table 2 lists common designs.

- **Biochemistry** [4,5]
  PepSets are used in biochemical studies as enzyme substrates, inhibitors, or ligands. Table 3 covers some of the possibilities.

Table 1: Study of Epitopes

Purpose	Cytotoxic T-Cells	Helper T-Cells	Antibodies
Peptide Length (No. of Amino Acids)	9 to 20	13 to 25	6 to 20
Number of Overlapping Amino Acids	Not less than 8	Not less than 12	Not less than 5
N-terminus	Free amine	Free amine	Biotinylated with spacer, or acetylated
C-terminus	Free acid	Free acid or amide	Amide, or noncleavable

Table 2: Study of Pharmacologically Active Peptides

Purpose	Primary Scanning	Narrowing Down	Analoging (SAR)
Peptide Length (No. of Amino Acids)	5 to 20	Reduce by one AA from each end	Replace one AA at a time
Number of Overlapping Amino Acids	4 to 19	100%	100%

- **Molecular Biology** [6,7]
  Peptide libraries can be used to look for the sites of protein-protein or protein-nucleic acid interaction. Testing can be by direct capture of the protein or nucleic acid, by measurement of captured peptide, or by inhibition of another known binding interaction (Table 4). Protein sequences can be screened for interaction sites by making panels of antipeptide antibodies covering the whole protein (Table 5).

- **Immunodiagnostic Test and Vaccine Development** [8,9]
  Preliminary screening of all possible sites for effectiveness of antipeptide antibodies in a disease model can lead directly to the best vaccine candidates and save years of expensive "trial and error" research based on predictive methods (Table 5). Likewise, immunodiagnostic tests need to use peptide which is recognized most frequently and strongly by immune sera or cells (Table 1).

- **Medical and Veterinary Clinical Immunology Studies** [10,11,12]
  Understanding the basis of clinical disease related to the immune system, whether arising from protective immunity, immunopathology, transplant rejection or reaction to mutated cancer cells may require testing of large numbers of individuals over many proteins or epitopes. This may only be possible with peptide libraries, and to achieve the broad surveys needed, it is critical to use peptide reagents with the right characteristics (Table 1). When epitopes become defined through the use of libraries, frequently the next step is to work with purified peptides. Mimotopes purified custom peptide products can be supplied to a quantity and purity specification suitable for most projects and budgets.

Table 3: Peptide Libraries for Biochemical Studies

Application	Peptide Design Feature
Protease substrate	Reporter groups (FRET, fluorescence polarization)
Phosphorylation substrate	Capture group or tag
Inhibitor library	Unnatural amino acid, nonpeptide bond or chemical grouping
Binding ligands	Reporter or capture group

- **New Drug Discovery** [13]
  Peptide Libraries can be a rich source of candidates for a drug development program. For example, an SAR study on a bioactive peptide fragment can reveal the critical residue(s) for bioactivity, which can then be further "analoged" to obtain more potent leads and peptidomimetics.

Table 4: Peptide Libraries in Interaction Screening

Measurement Method	Peptide Design Feature
Direct capture of macromolecule	Biotinylated or noncleavable peptides
Measure captured peptide	Biotinylated or fluorescent peptide
Inhibit known interaction	No peptide modification required

Table 5: Antipeptide Antibodies as Probes

Design Feature	Specification
Peptide length (No. of Amino Acids)	8 to 24
Number of overlapping Amino Acids	7 or more
N-terminus	Conjugation group (sulfhydryl) + biotin
C-terminus	DKP (linker)
Method of use	Simultaneous cleavage and conjugation

Choosing the Right PepSets Design

Staff at Mimotopes have been designing, synthesizing and testing PepSets for immunological and other applications since 1982. Mimotopes offers an obligation-free design assistance service, which will include assessment of the feasibility of the peptides and a quotation for the specific set as designed for you.

After Mimotopes technical consultants have gained an understanding of the needs of your project, they will be able to recommend a strategy to create the most economical PepSet design for you, and will assist in the creation of the list of peptide sequences. If your needs are particularly complex or innovative, Mimotopes biologists and chemists will offer solutions tailored to your needs.

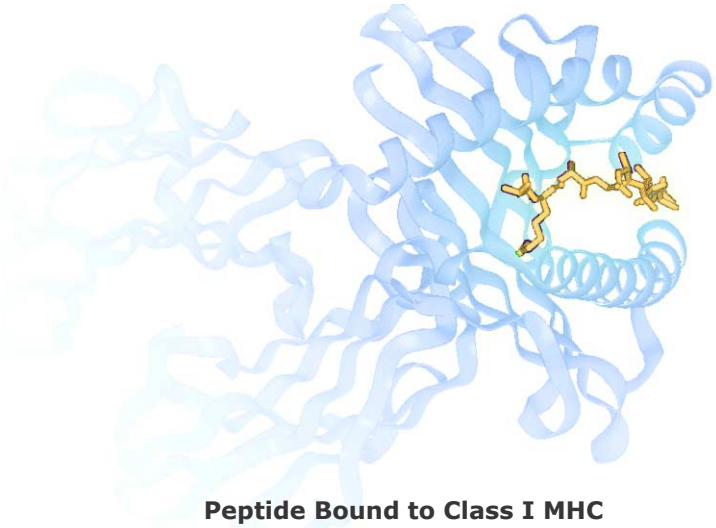
Alternatively, you can design and specify the peptides, and Mimotopes, after confirming their feasibility, will manufacture them to your design. Synthesis will normally commence immediately upon order placement, and delivery takes around four weeks, varying a little with peptide length and total number of peptides. PepSets can be ordered directly from Mimotopes, or through Mimotopes sales offices in various countries, or through our network of distributors. Specific order conditions will be included in the quotation.

Mimotopes' commitment is to provide technical support to you throughout your PepSet application work.

Getting Results with PepSets

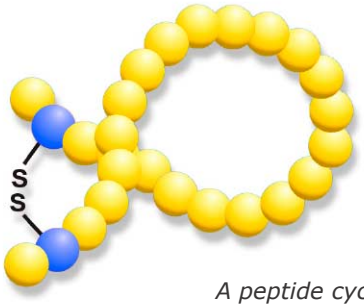
Mimotopes can assist you to obtain fast and efficiently generated results with your PepSet. For example, the first process followed by most Mimotopes Cleaved PepSets users is usually to redissolve all the PepSet peptides, which are supplied as dried powders in individual capped tubes. For projects where direct binding tests are going to be carried out, Mimotopes offers biotinylated peptides which have already been taken to the next step, capture onto streptavidin-coated microtiter plates in an array layout. Prior coating of biotinylated peptides onto multiple streptavidin-coated microtiter plates saves significant time, effort and additional expense in the user's hands. These plates can be immediately used for tests of direct binding, for example in an antibody epitope mapping project, and the resulting data can be obtained within a day of receiving the peptide-coated plate product.

Even more convenient is the full antibody epitope mapping service offered by Mimotopes, where the coating and ELISA testing are carried out at Mimotopes' laboratories, and results are QA checked before forwarding to you.

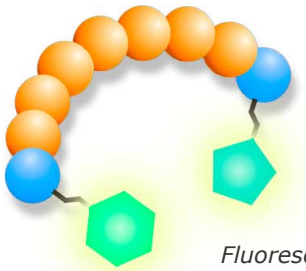


Peptide Bound to Class I MHC

Using a PepSet is the ideal way to test a wide range of peptides for Major Histocompatbility Antigen binding or T-cell activation.



A peptide cyclized through a disulfide bond between two cycsteine residues.



Fluorescence Resonance Energy transfer (FRET) peptide libraries are ideal for protease specificty mapping.

Examples of available peptide library designs

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