Biotinylated PepSets™

Libraries of biotinylated peptides are ideal for rapid scanning of protein sequences for bioactive sites

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Epitope Mapping the Fast Way

Custom-synthesized peptide libraries (PepSets) from Mimotopes are the quickest and easiest way to obtain bioactive peptide data in a wide range of bioresearch areas: Antibody and T-Cell Epitope Mapping, Immunodiagnostic Test and Vaccine Development, Medical and Veterinary Clinical Immunology Studies and peptide-related Drug Discovery. This article focuses mainly on linear antibody epitope mapping with biotin-labelled peptides from Mimotopes.

The PepSets peptide libraries are custom synthesized on a small scale to cover all the possible sequences from the protein of interest. All the peptides, made on a small scale and used in the unpurified form, are screened in parallel. Positive results are then confirmed with characterized peptide.

Linear Antibody Defined Epitope Mapping

Linear antibody epitopes are readily mapped with PepSets. Sets of overlapping peptides from potential target vaccine proteins are screened with immune sera or cells to find vaccine or diagnostic test candidates. This saves years of expensive "trial and error" research based on predictive methods.

Antibody epitope mapping by ELISA is simple to perform using biotinylated peptides because the peptides can be captured onto Streptavidin™ or NeutrAvidin™ coated plates through the strong noncovalent avidin-biotin binding. Probing the wells with antibody, and washing away unbound antibodies, quickly reveals those peptides to which the antibody has bound. For simplicity of reagents, an indirect ELISA is usually preferred so that a common enzyme-labelled reagent can be used for all tests (Figure 1).

In a typical example, a library of monoclonal antibodies to a small protein, thought to recognize linear epitopes, was screened using a PepSet of biotinylated peptides (Figure 2). The set consisted of short overlapping peptides derived from the protein being investigated and the peptides were captured onto plate wells coated with streptavidin before completing an ELISA with each monoclonal antibody (Figure 1). Using this approach, the linear epitopes of several monoclonal antibodies were quickly and clearly identified. The monoclonal antibodies fell into groups recognizing one of three linear sequences of the protein. One example from each group is shown in Figure 3.

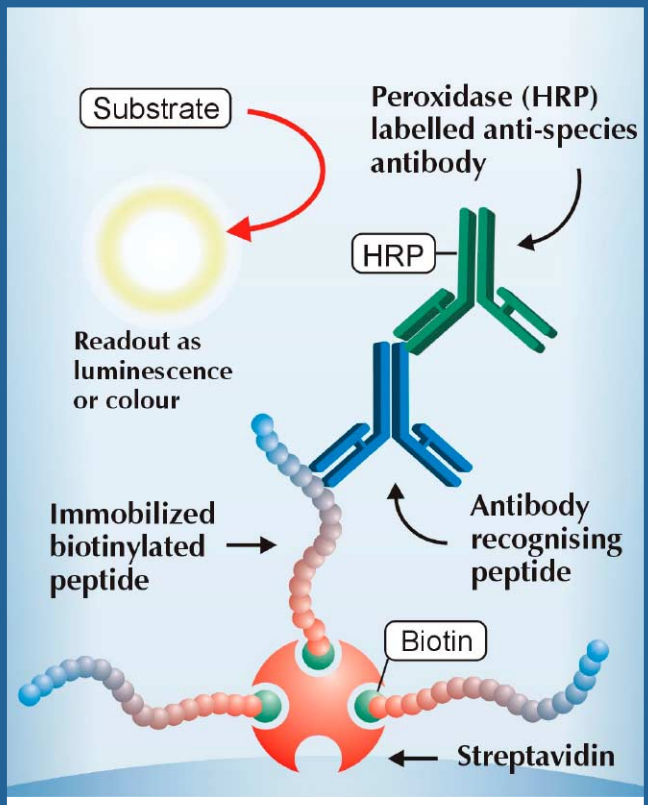

Figure 1: An ELISA using biotinylated peptides and coated plates

Advantages of Biotinylated PepSets for Mapping

PepSets have several advantages over other screening methods such as noncleaved peptides and blots. These are:

- **High throughput**
  Simultaneous parallel testing of all the antibodies for immediate results
- **No re-probing**
  All tests are performed with new peptide
- **Quantitative data**
  OD readings from a standard lab plate reader, versus image analysis etc.
- **Lower backgrounds**
  When compared with membrane- or card-bound peptide
- **Minimal steric hindrance to binding**
  Due to the spacer incorporated between the biotin and the peptide[1].
- **Essentially unlimited numbers of tests from one PepSet**
  The 1-3mg scale PepSet is enough peptide for several thousand ELISA tests.

Customer Service and User-friendly Products

Custom synthesized biotinylated PepSets for linear antibody mapping are designed by the research scientist with help from experienced Technical Consultants at Mimotopes, and are shipped either ready to redissolve and plate or as ready-to-use plates already coated with the peptide (Figure 2).

Other Powerful PepSets Applications

T-cell epitopes (Helper and Cytotoxic) are easily located using PepSets provided peptides of the correct length are used[2]. This application is summarized in a separate article entitled Truncated Peptide Libraries for Cytotoxic T-Cell Epitope Mapping, available on the Mimotopes website. When a bioactive sequence is already known, analoging studies can be carried out using the set of peptides comprising single-point substitution sequences, or a combination of 2 or more selected substitutions. Sequences exhibiting enhanced bioactivity, or inhibitory properties, are candidates for development as peptide or peptidomimetic drugs.

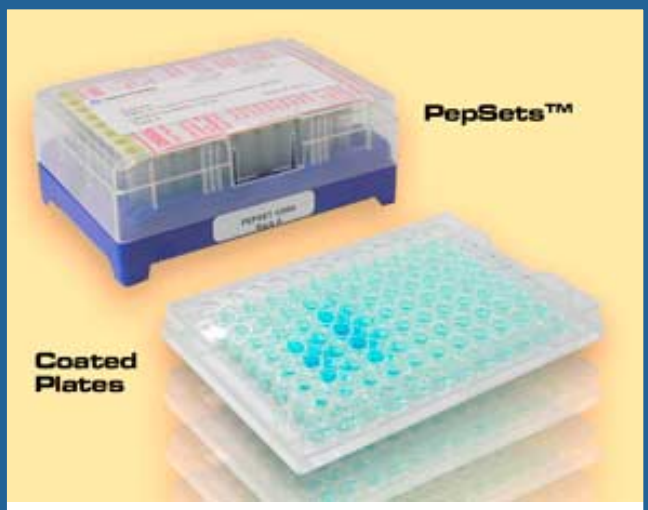

Figure 2: A packaged PepSets peptide library with coated plates

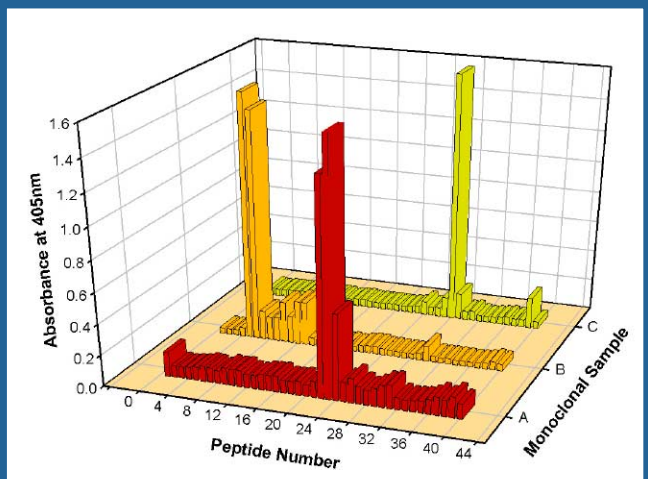

Figure 3: Elisa results with 3 monoclonal antibodies

Applications in Biological Research

Some of the most frequently used applications are listed below.

- **Immunology**
  The most common use of PepSets in immunology is for B and T-cell epitope mapping.

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

- **Biochemistry**
  PepSets are used in biochemical studies as enzyme substrates, inhibitors, or ligands.

- **Molecular Biology**
  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. Protein sequences can be screened for interaction sites by making panels of antipeptide antibodies covering the whole protein.

- **Immunodiagnostic Test and Vaccine Development**
  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. Likewise, immunodiagnostic tests need to use peptide which is recognized most frequently and strongly by immune sera or cells.

- **Medical and Veterinary Clinical Immunology Studies**
  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. 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.

- **New Drug Discovery**
  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.

References

1. Weiner, A. J., Geysen, H. M., et al. (1992) Evidence for immune selection of hepatitis C virus (HCV) putative envelope glycoprotein variants: Potential role in chronic HCV infections. Proc. Natl. Acad. Sci. USA 89; 3468-3472.

2. Rodda, S.J. (2002) Peptide Libraries for T-Cell Epitope Screening and Characterization. J. Immunological Methods, 267, 71-77.

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