



proteomics

Peptide Antigen Design for Antibody Production

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Application Notes

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- Antigen design
- Peptide synthesis and conjugation
- 77-day immunization and bleed protocol
- Variety of hosts
- ELISA and sera purifications
- Phosphospecific antibody production

Introduction

Antibodies to small 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, and active site determination. Design of the antigen used for eliciting an immune response is a critical factor in antibody production. This application note will briefly explore peptide selection and design, coupling strategy, and carrier proteins, which are important factors in anti-peptide antisera generation.

Peptide selection and design

When examining a protein sequence for potential antigenic epitopes, it is important to choose sequences that are hydrophilic, surface oriented, and flexible. Most naturally occurring proteins in aqueous solutions have their hydrophilic residues on the surface and their hydrophobic residues buried in the interior. Antibodies bind to epitopes on the surface of proteins. The N- and C-termini of proteins are often exposed and have a high degree of flexibility, often making these regions a good choice for generating anti-peptide antibodies directed against the intact protein.


Algorithms for predicting protein characteristics such as hydrophilicity/hydrophobicity and secondary structure regions such as α -helix, β -sheet and β -turn aid in selection of a potentially exposed, immunogenic internal sequence for antibody generation. Surface regions or regions of high accessibility often border helical or extended secondary structure regions. In addition, sequence regions with β -turn or amphipathic helix character have been found to be antigenic.

Peptide length also should be considered when designing your antigen. The typical length for generating anti-peptide antibodies is in the range of 10-20 residues. Peptide sequences of this length minimize synthesis problems, are reasonably soluble in aqueous solution and may have some degree of secondary structure. Sequence epitopes in proteins generally consist of 6-12 amino acids in length.

Coupling strategy

The most overlooked factor when designing a synthetic peptide is the method of coupling the peptide to the carrier protein. It is important to ensure that the peptide is presented to the immune system in a manner similar to the way it would be presented by the native protein. For example, N-terminal sequences should be coupled through the C-terminal amino acid and *vice versa* for C-terminal sequences. Internal sequences can be coupled at either end. Another consideration for internal sequences is to acetylate or amidate the unconjugated end as the sequence in the native protein molecule would not contain a charged terminus.

The most common coupling methods rely on the presence of free amino (α -amino or Lys), sulfhydryl (Cys), or carboxylic acid groups (Asp, Glu or α -carboxyl). Coupling methods should be used that link the peptide to the carrier protein via the carboxyl- or amino-terminal residue. The sequence chosen should not have multiple residues that may react with the chosen chemistry. If multiple reactive sites are present, try to shorten the peptide or choose the sequence so they are all localized at either the amino or the carboxyl terminus of the peptide. For internal sequences, the end furthest from the predicted epitope is normally favored as this avoids potential masking problems.



The 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) method is used for coupling the peptide and the carrier protein via the amino or carboxyl group of the peptide sequence, depending on the activation strategy. Carbodiimides can activate the side chain carboxyl groups of aspartic and glutamic acid as well as the carboxyl terminal group to make them reactive sites for coupling with primary amines. The activated peptides are mixed with the carrier protein to produce the final conjugate. If the carrier protein is activated first (Activated EDC), the EDC method will couple the carrier protein through the N-terminal α -amine and possibly through the amine in the side-chain of Lysine, if present in the sequence.

The m-Maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) is a heterobifunctional reagent that can be used to link peptides to carrier proteins via cysteines. The coupling takes place with the thiol group of cysteine residues. If the chosen sequence does not contain Cys, it is common to place a Cys residue at the N- or C-terminus to obtain highly controlled linking of the peptide to the carrier protein.

Selecting the carrier protein

Conjugation to a carrier protein is important because peptides are small molecules, that alone do not tend to be immunogenic, thus possibly eliciting a weak immune response. The carrier protein contains many epitopes that stimulate T-helper cells, which help induce the B-cell response. The most commonly selected carriers are key-hole limpet hemacyanin (KLH) and bovine serum albumin (BSA). The higher immunogenicity of KLH often makes it the preferred choice. Another advantage of choosing KLH over BSA is that BSA is used as a blocking buffer in many experimental assays. Because antisera raised against peptides conjugated to BSA will also contain antibodies to BSA, false positives may result. Ovalbumin (OVA) is another useful carrier protein. It is a good choice as a second carrier protein when verifying whether antibodies are specific for the peptide alone and not the carrier. Rabbit Serum Albumin (RSA) may be used when the antibody response to the carrier protein must be kept to a minimum. Rabbits immunized with RSA conjugate are less likely to raise antibodies to the carrier, as the RSA is recognized as "self". It is important to recognize that the immune system reacts to the peptide-protein carrier as a whole and that there will be a portion of response directed against the peptide as well as the linker and the carrier protein.

Choice of host

When attempting to raise an antibody, choose an animal that is genetically very different from the source of the immunogen. In order to achieve maximum immune response, it is important to avoid self-recognition of the immunogen by the host animal. As an example, it is more suitable to use a rabbit or mouse host than a monkey. For highly conserved mammalian proteins, raising antibodies in the avian (chicken) system is often a preferred alternative.

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