

Peptides for Immunization

Description

Antibodies against specific peptides are commonly used in research, diagnostics and medical therapy.

Peptides and other small molecules that are used as antigens are called haptens. They are able to act as triggers for production of specific antibodies, but cannot stimulate the necessary immune response themselves. Peptides are incomplete immunogens due to their small size. Coupling to a suitable carrier molecule can turn them immunogenic. Another approach is to increase the size by combining multiple peptide units.

The most commonly used peptide modifications for use in antibody production are:

- Conjugation to carrier proteins
- Multiple antigenic peptides (MAPs)

Protein Conjugates

Peptide-protein conjugates are used for antibody production against peptides. Typically, peptides alone are too small to elicit a sufficient immune response. Therefore, carrier proteins, such as KLH, BSA and OVA, that contain many epitopes are used to stimulate T-helper cells, which help induce the B-cell response.

It is very important to remember that the immune system reacts to the peptide-protein conjugate as a whole, so there will always be a portion of antibodies to the peptide, the linker and the carrier protein.

The choice of conjugation chemistry depends on the functional groups available on the hapten, the required hapten orientation and distance from the carrier, and the possible effect of conjugation on biological and antigenic properties.

There are two main methods for coupling peptides to carrier proteins:

1) Coupling of terminal cysteine containing peptides via their thiol groups to maleimide activated carrier proteins¹⁾

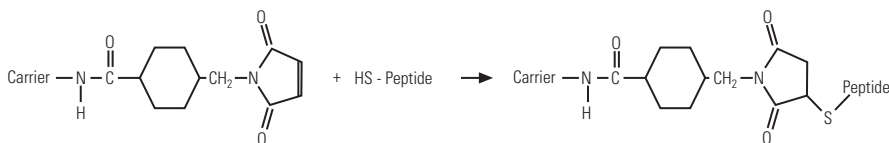
- most favorable method for specific antibody formation
- defined orientation of the antigen
- minimized influence on the antigenic structure
- terminal cysteine required (if not already present, cysteine can be added at the N- or C-terminus)
- additional internal cysteines should be avoided
- possibility of antibody formation against linker

2) Coupling of carboxyl containing peptides to primary amino groups of the carrier²⁾

- no cysteine required
- no additional linker, causing undesired antibody formation
- lower antigen density
- many different steric conformations due to different binding ways and possible peptide polymerisation
- possibility of reduced solubility

1)

Maleimide mediated conjugation:



2)

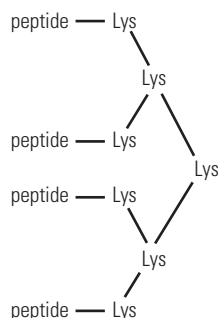
EDC mediated peptide carrier conjugation:



Most common carrier proteins

- KLH (Keyhole Limpet Hemocyanin) is a copper containing, non-heme protein found in arthropods and mollusca with a molecular weight of MW $4.5 \times 10^5 \sim 1.3 \times 10^7$ Da. It is normally isolated from *Megathura crenulata*. KLH is the most commonly selected carrier for immunization in vertebrata. KLH shows higher immunogenicity compared to BSA due to its higher molecular weight and high number of primary amines for coupling haptens. Highly activated KLH shows >400 binding groups per molecule, about 200–300 normally react with peptides.
- BSA (Bovine Serum Albumin) is a very stable and highly soluble plasma protein from cattle. It has a MW of 67×10^3 Da and contains 59 lysine residues. About 30-35 of these primary amines are accessible for conjugation, which makes BSA a popular carrier protein. A disadvantage of BSA is that it is used in many experiments as a blocking buffer reagent. If antisera against peptide-BSA conjugates are used in such assays, false positives can occur, because these sera also contain antibodies to BSA.
- OVA (Ovalbumin), a protein isolated from hen egg whites, with a MW of 45×10^3 Da. It is a good choice as a second carrier protein to verify antibodies that are specific for the peptide and not the carrier protein (e.g., BSA). Activated OVA show about 5-15 binding groups per carrier molecule for direct coupling of haptens.
- MAPs (Multiple Antigen Peptides)
MAPs are branched peptides that can be used for direct immunization to produce antibodies. MAPs are usually large enough to trigger immune response. The antigenic peptide of interest is synthesized directly on the branched linker structure. MAPs are available as MAP 4 (4 branches) or MAP 8 (8 branches) molecules.

Schematic graph of a MAP 4:



- fully chemically synthesized, free of biological contaminants
- no undesired antibody formation against carrier proteins
- defined orientation of antigen (coupling at the C-terminus)
- purification and analytics of MAP peptides are not possible (only crude MAP available)

Application

- peptide antibody production
- antibody purification
- antibody testing

Product offering

- *Peptides for Immunization Assays*

Conjugates to Carrier Proteins (KLH, BSA or OVA) available for peptides with 6 – 25 amino acids (aa)
Any remaining free peptide will be delivered at free of charge.

Possible peptide purity

70%	95%
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Final amount of conjugate

2 mg	5mg	10 mg
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Final amount of MAP

10 mg	20 mg	50 mg
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In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

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