

Maleimidyl activated Protein Carriers

Products Description

Protein carriers for convenient preparation of immunogens using cys containing peptides or Ags.

Maleimidyl KLH	86734A, 2mg	86734C, 10mg
Maleimidyl BSA	86695A, 2mg	86695C, 10mg
Maleimidyl OVA	23066A, 2mg	23066C, 10mg

Binding capacity: on mf of carrier will bind ~1-2 mg of peptide (average MW of 1000-2500)
 Provided in solution at 4mg/ml in PBS pH7.5.

Storage : -20°C, stable for 12 months – Stable 7 days at ambient temperature. ^(M)

Introduction:

Activated KLH, BSA and Ovalbumin (OVA), are commonly used as protein carriers in order to enable the immune response of small molecules, haptens such as peptides. In many cases KLH (or BSA) conjugate is preferred as immunogen for the immunization, while BSA or OVA conjugate is used as antigen in immunoassays to monitor the raised antibodies. Hence, using i.e. OVA conjugates filter out the antibodies directed to the KLH or BSA, to measure a more peptide-specific antibody response.

The **maleimide-based coupling procedure** used by these carriers offers several advantages over other used methods, such as glutaraldehyde or two step SMCC conjugation:

- **Fast - no activation required for the hapten**
the hapten should bear a SH group, but unlike SMCC method, no activation is required: only 2 steps: conjugation, and desalting
- **oriented peptide conjugation – no reticulation**
the maleimide reactivity is designed to get oriented coupling, with much more specificity than i.e. glutaraldehyde. No reticulation (intramolecular crosslinks) occurs in carriers.
- **efficient and reproducible conjugation**
the method ensures accurate conjugations, because 1/the activation ratio (maleimide content) is controlled 2/the reagents concentrations and incubations conditions are easier to set up, 3/ the maleimide reactivity is very fast and virtually complete.

Our Maleimide activated KLH, BSA and OVA are preactivated with a heterobifunctional cross-linker to graft maleimide groups. These activated proteins may be reacted with biomolecules that contain a free sulfhydryl groups, to form a stable thioether bond. As they are provide in stable solution, they are more convenient to use!: juste add your peptide!

Directions for use

1▪ Dissolve the sulfhydryl-containing hapten in a volume of water (or suitable buffer) equals to x0.4 to x1 times the volume of carrier (KLH, BSA or OVA).

For example dissolve 2 mg of peptide in 200-500 µl of buffer for addition to 2 mg of activated carrier in 500 µl.

Note: For haptens with limited solubility, DMSO may be used for solubilization. Use .30% DMSO in the final conjugation solution or the carrier protein may irreversibly denature.

Alkaline pH values (above 8.5) may hydrolyze the maleimide group or generate side reactions with amines. Haptens must contain cysteine or a sulfhydryl group in the reduced state in order to react efficiently with the maleimide group.

2▪ Thaw the Maleimide Activated carrier (KLH, BSA or OVA) at room temperature.

Note: Do not vortex or heat the activated CARRIER (KLH BSA OR OVA).

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3▪ Mix the peptide with activated carrier (KLH, BSA or OVA) adding peptide stepwise (1/4th of quantity each time in 5 minutes intervals) and watching for possible appearance of turbidity in solution.

If slight turbidity forms – stop adding peptide to KLH solution.

4▪ Reaction is done for 2 hours at room temperature under occasional tapping of tube.

5▪ Desalting, control, storage and use:

Peptide-conjugated KLH, BSA or OVA can be desalted by gel filtration, dialysis or any suitable purification method to remove coupling buffer. However, the conjugate usually do not need to be purified from non-conjugated peptide/hapten, nor from non-conjugated carrier (that is negligible).

The coupling efficiency of conjugation can be determined by assaying the content of free sulfhydryl groups in the unreacted peptide using DTNB reagent.

If the immunogen is to be stored for more than a few days it is recommended to store frozen at -20°C. preservatives may be added but should be removed before immunization. Also stabilizers such as glycerol may be added before freezing although usually not needed. EDTA should be absent for injection to animals because of its anti-coagulant effect.

The hapten-carrier conjugate can be used in your usual procedures of animal immunization with adjuvants (FIA/FCA,...), and in Ab screening assays (ELISA,...). Please ask Uptima if you need further information.

Technical and Scientific Information

- **Keyhole Limpet Hemocyanin (KLH)** is a large protein present in arthropods and molluscs, and typically prepared from hemolymph of giant keyhole limpets (*Megathura crenulata*). It is widely used as a carrier to bind haptens (small molecules not or poorly potent to raise immunization) and present them to the immune system: its large size and its non-mammalian related origin allow effectively strong immunogenicity and numerous coupling sites for haptens. **Bovine Serum Albumine (BSA)**, beside its numerous other uses, is also widely used as carrier for immunization, and furthermore, also conjugated to hapten, complementary to immunization with KLH, to detect raised specific Ab in screening assays.
- **Bovine serum albumin (BSA)**, also known as "Fraction V", is a serum albumin protein isolated from bovine blood. It is a protein of 66432 Da MW (40x40x140 cubic angstroms) (69KDa precursor form) with 607 residues. Its pI is 4.7 at 25°C and extinction coefficient 0.667 ml cm⁻¹. BSA has numerous biochemical applications, thanks to its stability, lack of biochemical reaction or for some specific properties, and because it is largely available and readily purified. Amongst these applications, BSA is used as a carrier protein for immunization, and for saturating unspecific binding sites or immunological supports (microplates wells, membranes, tissue slides, microarrays)
- **Ovalbumin** is the main protein found in egg white, making up 60-65% of the total protein. The function of ovalbumin is unknown, although it is presumed to be a storage protein in tissues (unlike its homology with the serpin superfamily, it is a non-inhibitory for serine proteases). The ovalbumin is a glycoprotein made up of 385 amino acids (relative molecular mass is 45 kDa) with 4 sites of glycosylation.
- Hapten or carrier conjugation procedures require chemical activation. Our **activated protein KLH, BSA and OVA carriers** provide a quick and simple method for the conjugation of cysteine-containing peptides to protein carriers KLH, BSA. Carrier-peptides can be used to immunize animals for antibody production, and for ELISA analysis of antibody response. Maleimide activated keyhole limpet hemocyanin (KLH) is primarily used for immunization, while Bovine serum albumin (BSA) is used for the titration of immunosera.
- The **maleimide group** reacts very specifically with sulfhydryls –SH at neutral pH 6.5-7.5. The reaction is rapid (a few minutes for cysteine), but in the absence of –SH, it is well stable. The hydrolysis forming maleimic acid becomes noticeable when pH goes up to 8.0, where the reactivity with amines begins to be possible. In usual conditions, one should start with a ratio of 10-20 moles of maleimide per mole of protein. With SH-peptides, a molar 1:1 incubation ratio allows almost 1:1 coupling.
- The molecule to be coupled (peptide, hapten, antigen) should contain **free sulfhydryl group(s) (SH)**. Cysteine-containing peptides provide a convenient method for such oriented conjugation. As free SH groups often oxidize in solution, SH content may be checked with DTNB assay, and oxidized cysteines (cystine, with –S-S- bridge) should be reduced before carrying out the conjugation step: this can be performed by any suitable reducer, but TCEP (#UP242214) is recommended because it offers several advantages (not pungent, very selective, does not reduce

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burried SH,...). Take care to desalt from excess reducing agent, or use alternative method using immobilized reducers.

Sulphydryls can also be incorporated chemically on primary amines with N-succinimidyl S-acetylthioacetate (SATA, Product #UP84235) or 2-iminothiolane.HCl (Traut's Reagent, Product #UP42425).

Maleimide activated protein carriers

Maleimide activated keyhole limpet hemocyanin (KLH) and Bovine serum albumin (BSA) are obtained by chemical activation to yield an optimal ratio of coupled maleimide groups. Both are supplied lyophilized in PBS pH7.5.

<i>Protein</i>	<i>Source</i>	<i>MW</i>	<i>form</i>	<i>ratio maleimide/Protein binding capacity (peptide 100-2500Da)</i>
KLH	Keyhole Limpet	4.5 10 ⁵ to 1.3 10 ⁷	solution 4mg/ml	~2-4 mg / mg KLH
BSA Albumine	Bovine serum	66.5 KDa	solution 4mg/ml	~2,5-4 mg / mg BSA
OVA Albumine	Chicken egg white	44.3 KDa	solution 4mg/ml	~2-4 mg / mg OVA

Other Information

Literature

Harlow, E., and Lane, D., eds. 1988. Antibodies: A laboratory Manual. Cold Spring Harbor Press.

Related / associated products

See [BioSciences Innovations catalogue](#) and [e-search tool](#).

* Other carriers and immunization reagents:

- . [TiterMax adjuvants](#) #R-1 and #G-1
- . Freund's Adjuvants, Incomplete [FCA] [#314335](#) and Complete [FIA] [#356155](#)

* Other conjugation methods:

- . ControlledAmine™ conjugation kit [#BL150A](#) (innovative conjugation, with stable and flexible activation of proteins)

* SMCC type crosslinkers: GMBS [#UP49608A](#) (less immunogenic);

- . MAL-PEO-NHS [#AL6580](#) (confers to conjugates more hydrophilicity and less immunogenicity)

* Purification:

- . Desalting products: CelluSep dialysis tubings (B101), FloatAlyse; desalting columns [#UP84874](#) (B132)
- . Antibody purifications tools (B166+)

For in vitro R&D use only

Please contact Uptima – Interchim for any other information

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