



Immunogens Preparation

[Peptide/Protein Conjugation for preparing Hapten- Carriers immunogens](#)

Featured products:

Peptide/Protein Conjugation kits

(KLH, BSA, OVA to Amino-, Thiol-, Carboxyl-containing haptens)

[Carriers & linker reagents](#)

Uptima Maleimide Activated KLH, BSA, and OVA

(Sulphydryl reactive)

[Adjuvants](#)

Freund's Adjuvant – M.tub.based

Titermax adjuvants – polymer-based

[Technical tips – Immunization basics](#)

Definitions – Useful carriers – Adjuvants



Peptide/Protein Conjugation for preparing Hapten-Carriers immunogen

BSA and KLH are general purpose preactivated carrier proteins for coupling peptide or other haptens. OVAAlbumin is used mostly to screening purpose.

■ Kits-for immunogen preparation using Maleimide-ACTIVATED CARRIERS

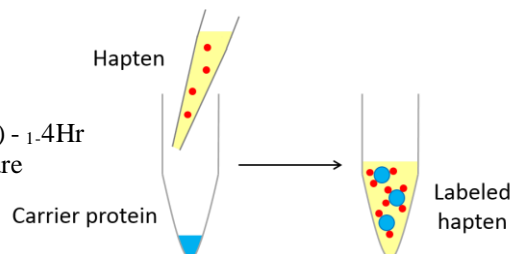
General purpose kits for coupling peptide or other haptens to preactivated carrier protein.

● Imm-Link™ Immunogen Kits

Conjugate small antigens (haptens) to carrier proteins (KLH/BSA/OVA) by 3 possible chemistries, which maximizes the immune response

- 3 chemistries Target Functional Groups
- Kit **Amine** Lysines or free amines (NH₂) - 2-4Hr procedure
- Kit **Carboxyl** Glutamic acid, aspartic acids or free carboxyls (COOH) - 1-4Hr
- Kit **Sulphydryl** Cysteines or free sulphydryl groups (SH) - 4Hr procedure

- Simple to use: mix hapten with the activator,
mix with carrier, conjugate is ready within 4Hr



● Imm-Link™ Immunogen Kits:

Kit KLH (for NH ₂)	450-0001, kit 2mg	450-0500, kit 3x2mg	Tech Sheet	
Kit KLH (for SH)	460-0001, kit 2mg	460-0500, kit 3x2mg	Tech Sheet	
Kit KLH (for COOH)	470-0001, kit 2mg	470-0500, kit 3x2mg	Tech Sheet	
Kit BSA (for NH ₂)	452-0001, kit 2mg	452-0500, kit 3x2mg	Tech Sheet	
Kit BSA (for SH)	462-0001, kit 2mg	462-0500, kit 3x2mg	Tech Sheet	
Kit BSA (for COOH) Tech Sheet	472-0001, kit 2mg	472-0500, kit 3x2mg	472-0015, kit 1x1mg	472-0010, kit 3x1mg
Kit Ovalbumin (for NH ₂)	451-0001, kit 2mg	451-0500, kit 3x2mg	Tech Sheet	
Kit Ovalbumin (for SH)	461-0001, kit 2mg	461-0500, kit 3x2mg	Tech Sheet	
Kit Ovalbumin (for COOH)	471-0001, kit 2mg	471-0500, kit 3x2mg	Tech Sheet	

Each kit contains:

- 1 or 3 glass vial(s) of Imm-Link™ mix
- 1 or 3 vial(s) of Imm-Link™ Amine Modifier reagent
- 1 or 3 dialysis cartridge(s)
- 1 or 3 250ml bottle(s) of 10X dialysis buffer

● Peptide/Protein Conjugation to Pre-Activated Carriers Kits

General purpose preactivated carrier protein (KLH) for coupling peptide or other haptens to KLH.

Description	Cat.number, Unit
PEPTIDE/PROTEIN CONJUGATION TO PRE-ACTIVATED KLH KIT	CDF070-80250 Pack : 1 Kit for 4 runs
Conjugate your proteins, peptides or antibodies to Heyhole Limpet Hemocyanin (KLH) for immunizations. Sufficient for 4 reactions	
PEPTIDE/PROTEIN CONJUGATION TO PRE-ACTIVATED BSA KIT	CDF060-80240 Pack : 1 Kit for 4 runs
Conjugate your proteins, peptides or antibodies to Bovine Serum Albumin (BSA) for immunizations. Sufficient for 4 reactions	
PEPTIDE/PROTEIN CONJUGATION TO PRE-ACTIVATED OVA KIT	CDF080-80260 Pack : 1 Kit for 4 runs
Conjugate your proteins, peptides or antibodies to Ovalbumin (OVA) for immunizations. Sufficient for 4 reactions	

.Kit contains: 1. 1 mg x 4 vials of pre-activated KLH, BSA or OVA;

Conjugation Buffer (20X), 25 ml #CB-20 (common to kit# 80240, #80250, and 80260 kits).

.Store kit at 4°C for 6-12 months.

[.Technical sheet](#)

Carriers & linker reagents

■ Uptima MaleimideACTIVATED CARRIERS (for immunogen prep.)

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

Maleimide activated BSA Albumine bovine activée au maléimide pour le couplage sur des sulfhydryles	86695A, 2 mg	86695B, 5 mg	86695C, 10 mg
Maleimide activated KLH Hémocyanine de Lamproie (Keyhole Limpet Hemocyanine) activée au maléimide pour le couplage sur des sSH	86734A, 2 mg	86734B, 5 mg	
Maleimide activated OVA Ovalbumine activée au maléimide pour le couplage sur des sulfhydryles		23066B, 5 mg	

Binding capacity: one mg of carrier will bind ~1-2 mg of peptide (average MW of 1000-2500)

Provided in solution at 4mg/ml in PBS pH7.5. [Fiche Technique^{\(1\)}](#)

■ Uptima Carriers and Crosslinker for Antigen - CARRIERS preparation

Popular carrier preparations are the glutaraldehyde method, the EDC (useful for peptides) conjugation, and the Maleimide-Succinimidyl oriented conjugation (useful for Cys-terminated peptides). Here is a selection of popular reagents.

•Carriers

BSA (Bovine Serum Albumin)	UP909383, 20 mg	Q84171, 100g (std grade)	71151A, 50ml(lowendotox.)
KLH (Keyhole Limpet Hemocyanin)	992335, 50 mg		
OVA (Ovalbumin)	R5851B, 5 mg		
Mollusk Hemocyanin CCH (immunogenic carrier)	88236A-, 500mg		

Concholepas concholepas hemocyanin (CCH). Increased solubility over KLH, with essentially the same level of immunogenicity.

•amine and thiol reactive (MAL-NHS oriented conjugation)

SMCC	UP34253A, 25mg	UP17412N, , 100 mg	1g
LC-SMCC	L7739A, 50mg	L7739B, 100mg	
SulfoSMCC	UP17412B, 50mg	UP17412A, 100 mg	1g
Maleimide-PEO₈-NHSuccinimide	, 100 mg	1g	
Maleimide-PEG₁₀₀₀Da-NHSuccinimide	, 100 mg	1g	
8-ARM-PEG₁₀₀₀Da-MALEIMIDE	B3AHC1, 1g		

•carbonyl reactive

EDC (EDAC)	UP52005A	UP52005B mg
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•glycoside reactive – click chemistry

SMCC-Hydrazide	BI1281, 100mg
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See description in the [BioScience catalog page C115](#).

Adjuvants

Freund's adjuvant

Freund's Complete Adjuvant (CFA)	356155, 6x10ml	356156, 50x10ml
Freund's Incomplete Adjuvant (IFA)	314335, 6x10ml	314336, 50x10ml

[Technical sheet](#)

Titermax gold adjuvants

safer and easier to use and at least as effective as Freund's Complete Adjuvant

- improved immunostimulatory activity of components
- contains block copolymers, squalene, a metabolizable oil, and a unique microparticulate stabilizer
- form a stable water-in-oil emulsion

Titermax gold	R-1, 6x2ml
Titermax	G-1, 6x2ml

[Technical sheet](#)

Technical tips – Immunization basics

[<https://en.m.wikipedia.org/wiki/Immunogen> article +++ car dit l'essentiel en 1page]

•Definitions

An **immunogen** is a compound that can by itself (as an antigen) or once presented in proper way (adsorbed to particles, bound to a carrier) induce a specific immune response (antibody, lymphocytes). An **antigen** is defined by its ability to be recognized specifically by immune system (an antibody or a T-lymphocyte), but not every antigen can evoke an immune response when exposed to an animal body (injected, inhaled,...). It however can become immunogenic once conjugated to another molecule this is itself immunogenic, or because of the size of the conjugate that allows sequestration in immuno-competent organs (lymph node) and suitable presentation to Antigen Presentation Cells (CPA).

The immunogenicity is so the ability to induce a humoral and/or cell-mediated immune response rather than immunological tolerance. A

In biotechs, an immunogen is either a large antigen, or a complex or conjugate of non immunogenic (small) molecule (called **hapten**) with a macromolecule (called **carrier**). The hapten-carrier complex, unlike free hapten, can act as an immunogen and can induce an immune response. The very minute part(s) of the hapten, antigen, or immunogen, that is recognized by a defined antibody is called **epitope(s)** (determinants), which typically covers 4-6 aminoacids in a protein.

•Useful carrier proteins

• Keyhole Limpet Hemocyanin (KLH)

KLH is a copper-containing respiratory protein, isolated from keyhole limpets (*Megathura crenulata*), that is very immunogenic in vertebrate animals because of its evolutionary distance from mammals, high molecular weight and complex structure.

• Concholepas Concholepas Hemocyanin (CCH) ()

CCH is isolated from *Concholepas concholepas*. It has the similar immunogenic properties as KLH but better solubility and therefore better flexibility.

• Bovine Serum Albumin (BSA)

BSA is isolated from the blood sera of cows. It has good immunogenic properties in general, and its large availability and high solubility make it a popular choice for use as carrier for immunizations. Antibodies raised against BSA itself may in some applications be a drawback (with polyclonal antibodies).

• Ovalbumin (OVA)

OVA, or egg albumin, is the main protein (60-75%) found in hen egg white. It is soluble in Dimethyl Sulfoxide (DMSO), which enables the conjugation of haptens that are not soluble in aqueous buffers. Its lower size makes it less immunogenic, so the immune response can be enhanced using an adjuvant injected together with the immunogen. A wise application is to use OVA for haptens that are difficult to use (immobilize) in testing applications: hapten-OVA conjugates are used in such tests of immunosera raised with BSA-hapten or KLH-hapten, so anti carrier(BSA,KLH) antibodies are not detected.

•cationized forms of carrier, i.e. of BSA (cBSA)

Cationization of carrier proteins converts their amines to carboxyls, creating a highly positively charged protein. Cationized carriers can subsequently be conjugated to more haptens, and have in general increased immunogenicity.

Immunological adjuvants

An **adjuvant** (from Latin adjuvare – to help) is a substance that, injected with immunogen, enhances the immune response by various mechanisms:

- recruiting of professional antigen-presenting cells (APCs) to the site of antigen exposure;
- increasing the delivery of antigens by delayed/slow release (depot generation);
- immunomodulation by cytokine production (selection of Th1 or Th2 response);
- inducing T-cell response (prolonged exposure of peptide-MHC complexes [signal 1] and stimulation of expression of T-cell-activating co-stimulators [signal 2] on the APCs' surface) and targeting (e. g. carbohydrate adjuvants which target lectin receptors on APCs).

Aside adjuvants used in vaccine medicine, adjuvants are used in biotech to boost the immune response. The most popular adjuvant is surely Complete and Incomplete Freund's adjuvant, solutions of aluminum hydroxide or aluminum phosphate. But many sophisticated adjuvants have been proposed, including polymers alternating hydrophilic and hydrophobic motifs.

Related products/documents

[Products HighLights Overview](#), including:

[Crosslinking tools](#) – PEO/PEG biotinylation agents and AmineControlled conjugation kit

[FluoProbes labeling agents](#)

[Dialysis and Desalting tools](#) – CelluSep tubings, SpectraPor tubings, GebaFlex, FloatALyser, SlideALyser,...

Information inquire

Reply by Fax : +33 (0) 4 70 03 82 60 or email at interbiotech@interchim.com

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