

Antigen preparation for Immunization

Interchim provides :

- ◆ Standard adjuvants (Freund adjuvant), as well innovative adjuvants for specific applications (TiterMax)
- ◆ Activated protein carriers and conjugation kits for antigen-carrier preparation

Carriers proteins

The choice of carrier should be based on :

- ◆ Its own ability to stimulate the immune response
- ◆ Its ability to be adequately coupled to the desired hapten

Keyhole Limpet Hemocyanine (KLH) is a classic and efficient used carrier for small haptens. Bovine serum albumin and Ovalbumin are good carriers too. MaxiBind BSA OVA bLG are improved carriers for creating multivalent antigens.

BSA Highest Purity, Powder

Suits Molecular Biology for the most exigent applications, immuno or genomic reagents stabilization. Can be used as carrier protein to create immunogens.

Purity >99%

DNAase, RNase, Ig Free

pH 5-5.4(% in water)

Cold ethanol fractionation

US origin

Description	Cat.#	Qty
BSA Highest Purity, Powder	UP909382	10 g

Ovalbumin

The major protein of egg white

Glycoprotein, MW 45 000 Da

Purity > 98%

Soluble up 50 mg/ml in water

Ovalbumin is used as carrier protein to create immunoconjugates with peptides. Beside excellent immunogenicity, conjugates are also used for immunoassays, notably to discriminate anti carrier antibodies.

Ovalbumin may also be used as control protein (molecular weight marker, irrelevant protein...), for coatings saturation, and for research purposes (protein interactions...).

Description	Cat.#	Qty
Ovalbumin, from Hen Eggs Powder	UPR5851A	1 g
	UPR5851B	5 g

KLH

Keyhole Limpet Hemocyanin from Megathura crenulata

90% of non-dissociated Hemocyanin

Copper/protein Ratio : 0.00249

Contains a proprietary preservative agent

Purity (exclusion chromatography) : > 90%

Solubility : 5-10 mg/ml in saline buffer

KLH stimulates very efficiently the immune response compared to other carriers, thanks to its big size. Our Uptima KLH offers enhanced purity and solubility.

Description	Cat.#	Qty
KLH, in PBS	UP885021	100 mg
	UP885022	5 x 100 mg
KLH, in BES	UP835990	100 mg
	UP865991	5 x 100 mg

Technical tip

Antigen preparation

The preparation of antigens is a key step for antibody production. Antigens may be immunogenic by themselves, and directly injected, or more generally combined to adjuvants. Synthetic peptides have become very important source of antigens in research. Because they are usually poorly or not immunogenic, they should be coupled to carrier proteins. These conjugates are then used as standard antigens, and for performing ELISA to assay the antibody that is raised against the peptide.

See also :

BSA standard grade	UPQ84170
BSA308 solution	UP900130

Isolation/Modification/Labeling

Antigen preparation for Immunization (Carriers-Ag)

Maleimide activated protein carriers and conjugation kits

Following kits and activated carriers provide a quick and simple method for the cysteine-containing peptides conjugation to protein carriers KLH, BSA, and any protein of your choice.

Kits contains all essential assay components, and are designed to ensure reliable conjugations with minimal hands-on time. They use activated proteins have been grafted with maleimidyl groups, that are available separately :

Maleimide activated KLH is primarily used for immunization, while Bovine serum albumin (BSA) is used for the immunosera titration. Both are supplied at 10mg protein/ml in 0.1 M Sodium Phosphate, 0.15 M NaCl, 0.1 M EDTA, pH 7.2.

Carrier Specification : 50-200 maleimide/HLH,
 >50maleimide/BSA,
 >10maleimide/OVA

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

Description	Cat.#	Qty
Maleimide Activated KLH Conjugation Kit contains activated KLH and all essential components to conjugate ca 5 x 1mg peptide	88504A	1 kit
Maleimide Activated BSA Conjugation Kit contains activated BSA and all essential components to conjugate ca 5 x 1mg peptide	88505A	1 kit
Maleimide Activated Antigen Conjugation Kit contains activated BSA and KLH, and all essential components to conjugate ca 5 x 1mg peptide with both carriers.	HG3060	1 kit
Maleimidyl KLH	86734A	5 mg
Maleimidyl BSA	86695A	5 mg
Maleimidyl OVA	23066A	5 mg



Specifications :

MaxiBind™ proteins are modified chemically on carboxylic functions by diamine ethylene. This alteration introduces ammoniums, hiding negative charges of carboxylates and resulting in a significant increase of the Isoelectric Point (PI) ^[1].

The result is a "cationized" molecule essentially positive with reactive charges.

- ◆ Reactivity on :
 - => Aldehydes
 - => Carboxylic acids
- ◆ Optimal number of reactive amines
 - => Conjugates at a high rate of couplings
- ◆ Positive ionic charge :
 - => Easy internalization in immunocompetent cells
- ◆ High purity
 - => From R&D to manufacturing
- ◆ Batch to batch (CV <10%)
 - => Easy scale changes

MaxiBind™ Super Active Carriers

MaxiBind™ proteins offer a growing number of amine functions to participate to coupling reactions with haptens or macromolecules and to improve the presentation of conjugates to the immune system.

These proteins are therefore proving to be the best choice for many applications for the preparation of conjugates and particularly to obtain very immunogenic carriers-haptens that can be used for immunization tests.

Applications

- ◆ Antibodies production : Immunogenic conjugates :
MaxiBind™ carriers (usually BSA) improve and increase the quantity and quality of the immune response [1] [2] [3] [4]
 - Faster induction of the immune response (both cellular and humoral)
 - Higher quantity of specific induced antibodies
 - Improved affinity of antibodies
- ◆ Conjugated antigens for tests :
MaxiBind™ carriers track better your specific antibodies !
 - Ideal for ELISA coating to monitor immunization
 - Useful as tracer or inhibitor in competitive assays
 - Double screening : 2 or 3 carriers allow to analyze cross-reactivity toward carrier and linker ; notably useful for monoclonal abs.
- ◆ Ligand interactions Studies
MaxiBind™ provide cationized surfaces for molecules interaction assays
 - Pharmacology : drug-carriers
 - Pharmacokinetics (distribution, elimination..) : tracers
 - Studies of receptors : cell binding
 - Studies of the endocytose
 - Biocaptors : cationized surfaces, special coatings

Description	Cat.#	Qty
BSA MaxiBind™	UP84225A	2 mg
Cationized Bovine Albumin 68-69 KDa	UP84225B	5 mg
	UP84225C	10 mg
	UP84225D	50 mg

Main use of BSA MaxiBind™ :

Carriers / Immunogens
Conjugates for various studies

Ideal to prepare your immunogens for the antibodies production, especially against little immunogenic haptens, peptides,...[1] :

- Faster induction of the immune response (cellular or humoral)
- Higher quantity of specific induced antibodies
- Improved affinity of antibodies

=>A 100 % increase of the number of reactive amine functions against the native BSA (70 functions instead of 35).

Description	Cat.#	Qty
OVA MaxiBind™	UP98505A	2 mg
Cationized Ovalbumin 44-45 kDa	UP98505B	5 mg

Main use of OVA MaxiBind™ :

Carriers / Immunogens
Immunization tests
Conjugates for various studies

A classic carrier for your immunization test conjugates :

- Easy bind onto activated or non-activated ELISA microplates (screening of monoclonal antibodies, immunization follow up)
- Creates conjugates at a high hapten/protein coupling rate allowing the detection of weak concentrations (IgM, IgG...)

=>Ideal for low molecular weight haptens or for haptens which are difficult to coat

=>High detection sensitivity

Description	Cat.#	Qty
β-LG MaxiBind™	UP98506A	2 mg
Cationized β-LactoGlobulin	UP98506B	5 mg
	UP98506C	10 mg
	UP98506D	50 mg

Main use of β-LG MaxiBind™ :

Immunization tests
Conjugates for various studies

Ideal to prepare your immunogens for the antibodies production, especially against little immunogenic haptens, peptides,...[1] :

- The background is the lowest for some detection systems.
- Can be used for immunization when one fears the antibodies induction with an hapten/albumin complex (ionic or hydrophobes link)

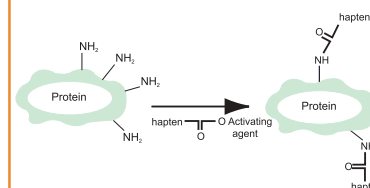
=>A carrier protein that is little known but which is very interesting

=>Ideal for the most applications demanding high specificity (double screening of monoclonal antibodies).

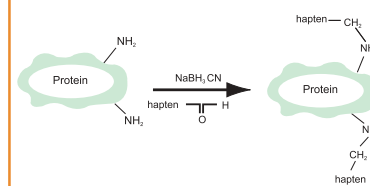
Technical tip

Principle of coupling

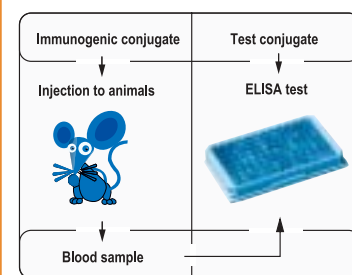
MaxiBind™ proteins / carboxylate function



MaxiBind™ proteins / aldehyde function



Protocol



- ◆ Dissolve the hapten in a minimum amount of coupling buffer
- ◆ Add EDC(#UP52005), and NHS (#UP04594)
- ◆ Add MaxiBind™ protein directly into the solution.
- ◆ Stir at room temperature overnight. Purify the hapten-carrier conjugate

[1] A. Muckerheide, P.L. Domen and J.G. Michael, Cationization of protein antigens. Alteration of immunogenic properties (1987). J. Immunol., 138, p. 833-837.

[2] F.S. Chu, H.P. Lau, T.S. Fan and G.S. Zhang. Ethylene diamine modified Bovine Serum Albumin as Protein carrier in the production of antibody against mycotoxins (1982). J. Immunol. Methods, 55, p. 73-78.

[3] R.J. Apple, P.J. Domen, A. Muckerheide and J.G. Michael. Cationization of protein antigens. Increased antigen uptake by antigen-presenting cells (1988). J. Immunol., 140, p. 3290-3295.

[4] R.N. Germain, The ins and out of antigen processing and presentation, (1986). Nature, London 322, p.687-689

Isolation/Modification/Labeling

Antigen preparation for Immunization (Carriers-Ag)

Adjuvants

TiterMax™ Immunization adjuvant / additive

A reliable and efficient alternative to the Freund's adjuvant !

=>No more batch variations, no more variable and difficult emulsions, no more difficult injections or severe inflammations on your rabbits ...

=>Titers often twice superior with one injection.

- ◆ Non toxic
Free of any proteins, peptides or bacterial/vegetal compounds
- ◆ Stable emulsion
Emulsifiers and stabilizers are chosen for a better ratio between efficiency and toxicity
- ◆ Efficient
High titers observed within 12 weeks without boost, thanks to proprietary immunomodulators
- ◆ Reliable
Synthetic and reproducible product, unique formulation

Description	Cat.#	Qty
Titer Max™ classic	R-1, 0.5 ml	5 x 0.5 ml
Titer Max™ gold	G-1, 1ml	5 x 1 ml

* Classic TiterMax™ contains copolymer CRL-8941 ; TiterMax™ Gold contains copolymer CRL-8300

Both classic TiterMax™ and TiterMax™ Gold suit many applications.

The one or the other is sometimes preferred because of particular performances according to the nature of the antigen or the immunization method.

Comparison between TiterMax™ / Complete Freund Adjuvant

	TiterMax™ adjuvants	Complete Freund adjuvants
Vehicle	Squalene	Mineral oil
Adjuvant	Modern Copolymer	Tuberculosis mycobacterium
Emulsifier	Non-ionic tensio-active	Non-ionic tensio-active
Stabilizer	Microparticules covered with copolymers	None
Emulsion formed	Water in oil	Water in oil
Security	Transitory inflammation	Severe transitory inflammation
Efficiency	The TiterMax™ without boost often exceeds the Freund with boost	Powerful adjuvant
Manufacturer	Strictly controlled manufacturing process	Variable quality according to manufacturers
Preparation	Similar procedure to Freund, creates a more stable emulsion, injectable with a needle	Little stable emulsion Difficult to inject

Isolation/Modification/Labeling

Antigen preparation for Immunization (Carriers-Ag)

Suggested dosage with TiterMax™ Classic (R-1)

Species	Method of injection	Number of injection	Volume of injection [a]	Injection place
Mouse	IM	2	20 µl	Each quadricep
	SC	1	40 µl	Base of the tail
Rat	IM	2	50 µl	Each quadricep
Guinea-pig	IM	2	50 µl	Each quadricep
	SC	4	50 µl	On each shoulder and each quadricep
Rabbit	IM	2	40 µl	Each quadricep
	SC	4	100 µl	On each shoulder and each quadricep
	ID	10	10 µl	Along the back
Goat/Sheep	IM	4	250 µl	2 injections in each quadricep
Chicken/	IM	2	50 µl	In each pectoral muscle
Turkey	SC	1	100 µl	In the neck
Horse/Cow	SC	10	100 µl	Along the neck

[a] Good responses were obtained with a relation water/TiterMax ranging from 10/90 to 90/10 but the ratio 50/50 is usually optimum.

ID : IntroDermal
IM : Intramuscular
SC : Subcutaneous

Suggested dosage with TiterMax™ Gold (G-1)

Species	Method of injection	Number of injection	Volume of injection [b]	Injection place
Mouse	IM	1	100 µl	Each quadricep
	SC	1	100 µl	Base of the tail
Rat	IM	2	100 µl	Each quadricep
Guinea-pig	IM	2	100 µl	Each quadricep
	SC	4	100 µl	On each shoulders and each quadricep
Rabbit	IM	2	100 µl	Each quadricep
	SC	4	200 µl	On each shoulders and each quadricep
Chicken/	IM	2	100 µl	In each pectoral muscle
Turkey	SC	1	200 µl	In the neck
Cat/Dog	IM	2	200 µl	Each quadricep
	SC	1	200 µl	Along the neck
Monkey	IM	2	200 µl	Each quadricep
Rhesus	SC	4	100 µl	Inner thigh
Goat/Sheep	IM	4	400 µl	2 injections in each quadricep
Horse/Cow	SC	10	200 µl	Along the neck

Some good responses were obtained with ratio water / TiterMax™ of 50/50.

Freund's Adjuvants

Freund's Adjuvants are non-specific stimulator of the immune response. When mixed with an antigen, they help to deposit or sequester the injected material. In addition, they cause a dramatic increase in the resultant antibody response. Freund's Complete Adjuvant is a water-in-oil emulsion and killed Mycobacterium, used for initial injections of antigen into animals to enhance the immune response, while the incomplete adjuvant does not contain Mycobacteria, and is used for further boosts. They are manufactured under controlled procedures for reproducible quality, of immunogenic grade. AFC/AFI has become a the most popular adjuvant, however with several limitations that have prompted the development of alternatives :

- 1/ it works well in most applications not for all antigens and/or not optimally,
- 2/ it elicits severe inflammations to animals,
- 3/ it may develop hypersensitivity to user.

Description	Cat.#	Qty
Freund's Adjuvants, Incomplete (FCA)	314335	6 x 10 ml
Freund's Adjuvants, Complete (FIA)	356155	6 x 10 ml

See also Hybridokine UP826430 (page E20)
A useful additive for immunizing mice and rat during monoclonal antibody development.