INSTRUCTIONS

Imject Maleimide Activated BSA and OVA

77115 77116 77125 77126

0135.4

Number	Description
77115	**Imject Maleimide Activated Bovine Serum Albumin,** 10mg
77116	**Imject Maleimide Activated Bovine Serum Albumin,** 2mg
	Note: Maleimide Activated BSA is lyophilized in a buffered solution containing 0.1M sodium phosphate, 0.15M sodium chloride, 0.1M EDTA, with stabilizer; pH 7.2.
77125	**Imject Maleimide Activated Ovalbumin,** 5 × 2mg
77126	**Imject Maleimide Activated Ovalbumin,** 2mg
	Note: Maleimide Activated OVA is lyophilized in a buffered solution containing 0.01M sodium phosphate, 0.01M EDTA, with stabilizer; pH 7.2.
	Storage: Upon receipt store product at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific™ Imject™ Maleimide Activated Carrier Proteins are activated using the crosslinker Sulfo-SMCC, which contains a maleimide group that is more stable to hydrolysis than maleimides of similar crosslinkers; only 4% of the maleimide groups of SMCC decompose at pH 7 at 30°C within 2 hours.

Ovalbumin (OVA) and bovine serum albumin (BSA) are conjugated to haptens and typically used as a non-relevant carrier in an ELISA for measuring specific anti-hapten antibody titers. Antibodies produced using mcKLH-hapten conjugates will recognize both the hapten and mcKLH. Coupling the hapten to a different carrier protein for the ELISA enables specific measurement of the anti-hapten antibody response. For more information on carrier proteins, see Appendix A.

Important Product Information

- Maleimide Activated BSA contains 15-25 moles of maleimide per mole of BSA; Maleimide Activated OVA contains 5-15 moles of maleimide per mole of OVA.
- Use a molar excess of peptide over the carrier protein's maleimide groups to ensure complete and efficient conjugation. For example, if the peptide's molecular weight is 2K, then add 2mg (1µmol) to 2mg of carrier protein (~0.7µmol of maleimide groups). Alternatively, if a molar excess of peptide is not available, after conjugation, add a sulfhydryl-containing compound, such as cysteine, to quench any remaining active maleimide groups.
- For hydrophobic peptides not soluble in the conjugation buffer, DMSO may be used, as it will not interfere with the reaction chemistry. Do not use greater than 30% DMSO in the final conjugation solution or the carrier protein may irreversibly denature. If DMSO is used in the conjugation, it may be necessary to add it to the buffer during gel filtration to prevent the conjugate from precipitating on the column. Do not use a higher pH buffer to solubilize the peptide as both maleimide hydrolysis and reaction with amines become significant above pH 8.
- Conjugation efficiency will vary from differences in the size and structure of peptides. The protocol is designed for the widest variety of applications, but is not necessarily optimal for a specific peptide. It may be possible to use less peptide and still obtain good results.
- The maleimide group reacts with SH groups but will not conjugate to disulfides. Failure to obtain conjugation may be a result of oxidation of SH groups on the peptide before or during conjugation. EDTA is included in the conjugation buffer to prevent metal-catalyzed oxidation of sulfhydryls.
- The maleimide activated carrier proteins are made from immunogen grade proteins and contain no preservatives.

General Procedure for Peptide-Carrier Conjugation

To quantify conjugation, compare sulfhydryl content before and after peptide conjugation by performing an Ellman's assay (Ellman's Reagent, Product No. 22582) on the peptide solution immediately after reconstitution (Step 2) and on the conjugate immediately after the reaction is completed (Step 3).

1. Reconstitute maleimide-activated carrier protein. Reconstitute Product No. 77115 and 77125 with 1mL of water and Product No. 77116 and 77126 with 0.2mL of ultrapure water to yield a solution containing 10mg/mL. Dilute with a buffer containing 0.1M sodium phosphate, 0.15M NaCl, 0.1M EDTA at pH 7.2 for concentrations < 10mg/mL.
2. Use up to 2mg peptide/2mg of activated carrier. Dissolve up to 2mg of sulfhydryl-containing peptide per 200-500µL of physiological pH phosphate buffer. Alternatively, add the peptide as a solid to the activated carrier solution if it is soluble.
3. Immediately mix the peptide and activated carrier solutions and allow them to react for 2 hours at room temperature.
4. To remove EDTA from the activated protein, purify the conjugate by gel filtration or dialysis. EDTA is an anti-coagulant and should not be injected into laboratory animals.

   Note: Dialysis minimizes sample loss and clogging of the desalting column when conjugating hydrophobic peptides. Desalting or dialysis will not separate BSA or OVA from the conjugated product; however, a large excess of peptide is used in this protocol, making it unlikely that non-conjugated carrier exists in significant quantity.
5. If the conjugate is to be stored for several days, sterile filter the conjugate fractions and store them in a sterile container at 4°C or frozen at -20°C. For extended storage, lyophilize the conjugate and store at 4°C.

Appendix

A. Carrier Proteins – Additional Information

Small molecules (haptens) such as peptides, although able to interact with products of an immune response, cannot stimulate a response. Haptens are incomplete immunogens but can be made fully immunogenic by coupling them to a suitable carrier molecule. Some of the more common carrier proteins include keyhole limpet hemocyanin (KLH; 4.5×10^5-1.3×10^7 MW), bovine serum albumin (BSA; 67,000 MW) and ovalbumin (OVA; 45,000 MW). KLH is the most widely used carrier because of its large molecular mass, strong immunogenicity and many available lysines.

mcKLH exists as five different aggregate states in Tris•HCl buffer. At pH 7.4, mcKLH reversibly dissociates to subunits with moderate changes in pH, while at pH 8.9 mcKLH completely dissociates to subunits. Each subunit contains oxygen-binding sites and, for every two copper atoms in mcKLH, one molecule of oxygen can bind. Removal of oxygen dissociates subunits into lower aggregation states. Increased antibody binding occurs when KLH is dissociated to its subunits because of improved availability of antigenic sites. Each mcKLH subunit is ~450,000 MW existing as a didecamer with an approximate molecular weight of 8×10^6. We formulate the mcKLH to dissolve quickly without the formation of precipitates that are often present in other commercial KLH products. Soluble mcKLH appears opalescent blue when dissolved.

Albumins, such as BSA, constitute approximately half of the protein in plasma and are the most stable and soluble proteins in plasma. BSA is much smaller than KLH but is also immunogenic and has an extinction coefficient of 7.16 at 280nm for a 1% solution. This protein has a total of 59 lysine groups with 30-35 of these capable of reacting with a cross-linker. BSA is often used for hapten conjugation and using the conjugate in ELISA to assay antibody generated by hapten coupled to KLH.

Ovalbumin contains 20 lysines and is often used as a secondary carrier in ELISA applications. This protein exists as a single polypeptide chain, with half of its 400 residues being hydrophobic. Ovalbumin has an acid isoelectric point of 4.63 and is subject to denaturation from vigorous shaking, temperatures above 56°C and electric current.

B. Immunization for Mice and Rabbits

After preparing the immunogen with an adjuvant (see Related Thermo Scientific Products section), the following protocol may be used for immunization (see Harlow and Lane, 1988). This schedule is a general protocol for immunization and may be customized as needed.

CAUTION: Only qualified personnel should perform immunization procedures. Individuals unfamiliar with these techniques should consult an experienced investigator for training before attempting to immunize and bleed animals.

Day 0: For mice, make initial injection of 50-100µg of immunogen per mouse (100-200µL of the emulsion) in one to two injections. The most common sites for injection into mice include intraperitoneal and subcutaneous.

For rabbits, collect non-immune serum to be used as a blank for ELISAs and store frozen. Make an initial injection of 100µg (200µL of emulsion) of immunogen into 6-10 subcutaneous sites on the animal's back.

Day 14: Boost with same sample size as the initial injection. If using Freund's adjuvant, use incomplete for boosts to avoid animal stress.

Day 21: Test bleed and measure for antibody response by ELISA. For mice, 200-400µL samples from the tail vein or the retro orbital plexis will be sufficient. For rabbits, 5-10mL samples from the ear vein are sufficient.

Day 28: Boost again if necessary. Continue to periodically bleed and boost until a satisfactory immune response occurs.

Related Thermo Scientific Products

77140	**Imject Freund's Complete Adjuvant,** 5 × 10mL
77145	**Imject Freund's Incomplete Adjuvant,** 5 × 10mL
77161	**Imject Alum,** 50mL
77605	**Imject Maleimide-Activated mcKLH,** 10mg
77661	**Imject Maleimide-Activated Blue Carrier Protein,** 2mg
43230	**Dextran Desalting Columns, 5K MWCO,** 5 × 5mL
22582	**Ellman's Reagent,** 5g
23225	**Pierce™ BCA Protein Assay Kit**

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