INSTRUCTIONS

Imject® BSA, OVA and mcKLH

In Phosphate-based Buffers

77110 77120 77600

0117.4

Number	Description
77110	**Imject BSA (in PBS),** 5 × 20mg
77120	**Imject OVA,** 5 × 20mg
77600	**Imject mcKLH (in PBS),** 5 × 20mg

Supplied: Carrier proteins are supplied lyophilized. When reconstituted with 2mL of ultrapure water the buffer compositions for BSA and mcKLH is 0.1M sodium phosphate, 0.15M NaCl; pH 7.2 and for OVA is 0.01M sodium phosphate, pH 7.2 . Proteins contain proprietary stabilizers.

Storage: Upon receipt store product at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific Imject Bovine Serum Albumin (BSA; 67kDa), Ovalbumin (OVA; 45kDa) and Mariculture Keyhole Limpet Hemocyanin (mcKLH; 4.5×10^5-1.3×10^7 Da) can be used for hapten conjugation for eliciting an immune response and antibody production against the hapten. OVA and BSA conjugated to haptens are also used as irrelevant carriers in an ELISA for measuring 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.

These carrier proteins are supplied in an ideal buffer formulation for conjugation using NHS-ester chemistry. NHS esters react with primary amines at pH 7-9 to form covalent amide bonds. Heterobifunctional crosslinkers that contain a NHS ester and a sulfhydryl-reactive maleimide group, such as Thermo Scientific Sulfo-SMCC (Product No. 22322), enable site-directed hapten conjugations. By reacting the crosslinker to the amines on the carrier protein first and then to a peptide containing a terminal cysteine, all peptide molecules will be coupled with the same orientation.

Procedure for Maleimide Activation of the Protein Carrier and Hapten Conjugation

A. Additional Materials Required

- Sulfo-SMCC (sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate) (Product No. 22322) – other crosslinkers, such as Sulfo-EMCS (Product No. 22307) or Sulfo-GMBS (Product No. 22324), also may be used
- Conjugation buffer, such as Thermo Scientific Imject Maleimide Conjugation Buffer (Product No. 77164), which contains 83mM sodium phosphate, 0.1M EDTA, 0.9M NaCl, 0.02% sodium azide, pH 7.2, with a proprietary stabilizer
- Desalting column (e.g., Thermo Scientific Polyacrylamide Desalting Columns, Product No. 43240) or dialysis cassettes (e.g., Thermo Scientific Slide-A-Lyzer Dialysis Cassettes, Product No. 66810)
- Sulfhydryl-containing hapten, 20mg
- Thermo Scientific Imject Purification Buffer Salts (Product No. 77159), upon reconstitution this buffer contains 0.083M sodium phosphate, 0.9M NaCl, with a proprietary stabilizer (see note below)

  Note: If the conjugate is to be used for injection within one week, PBS may be used instead of the Purification Buffer Salts for desalting. If the conjugate will be frozen, using the Purification Buffer Salts for desalting will preserve the conjugate during freeze-thaw cycles.

B. Maleimide Activation of the Carrier Protein

1. Reconstitute one vial of carrier protein by adding 2mL of ultrapure water to make a 10mg/mL solution.

   Note: mcKLH forms a suspension that typically appears translucent to whitish blue. Do not vortex or heat the suspension, which will cause the mcKLH to precipitate.

2. Immediately before use prepare a ~10mM solution of Sulfo-SMCC in ultrapure water (5mg/mL). Add the appropriate volume of Sulfo-SMCC to the carrier protein as follows: 2mL for mcKLH; 3mL for BSA; 4mL for OVA.
3. Incubate for 60 minutes at room temperature or 30 minutes at 37°C with periodic gentle mixing.
4. Use a desalting column equilibrated with Conjugation Buffer to remove excess crosslinker.

C. Hapten Conjugation

Note: The protocol is designed to yield effective immunogens for a wide variety of haptens but is not necessarily optimal for a specific hapten. Differences in size and structure of haptens will affect conjugation efficiencies. Using a molar excess of hapten over the carrier protein ensures efficient conjugation. Generally, reacting equal mass amounts of hapten and carrier protein will achieve sufficient molar excess. If a molar excess of hapten is not available, add a sulfhydryl-containing compound, such as cysteine, after conjugation to quench any remaining active maleimide groups.

1. Dissolve up to 20mg of the sulfhydryl-containing hapten in 5mL of Imject Maleimide Conjugation Buffer. 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.
2. Immediately mix the peptide and activated carrier protein and react for 2 hours at room temperature.
3. Purify conjugate by desalting or dialysis to remove EDTA and sodium azide.

Additional Notes:

- PBS may be used for conjugate purification. If the conjugate will be frozen, use the Purification Buffer Salts, which will preserve the product during freeze-thaw cycles.
- Desalting or dialysis will not separate non-conjugated protein; however, a large excess of hapten is used in this protocol, making it unlikely that non-conjugated carrier exists in significant quantity.
- If DMSO was used in the conjugation, add DMSO to the Purification Buffer Salts for desalting to prevent precipitation in the column; dialysis is not compatible with DMSO.
- If a precipitate has formed during conjugation, centrifuge the material, collect the supernatant and save the precipitate. Use only the supernatant for purification. Combine the purified conjugate to the precipitate.
- To purify antibodies specific to the peptide, prepare an affinity column by immobilizing the peptide through the same functional group used to prepare the immunogen. The Thermo Scientific SulfoLink Coupling Resin (Product No. 20402) contains an activated gel support that will couple peptides via sulfhydryl groups. The peptide affinity column can then be used to specifically bind anti-peptide antibodies from serum, allowing antibodies against the carrier protein to flow through the column. Peptide-specific antibodies can then be eluted and recovered.