INSTRUCTIONS

Imject™ BSA and mcKLH (in MES buffer)

77171 77653

0837.3

Number	Description
77171	**Imject BSA (in MES buffer),** 2 mg, supplied lyophilized; buffer contains 0.1 M MES, 0.15 M NaCl; pH 4.7 and sorbitol when reconstituted with 0.2 ml of water **Storage:** Upon receipt store product at 4°C. Product is shipped at ambient temperature.
77653	**Imject mcKLH (in MES buffer),** 2 mg, supplied lyophilized; buffer contains 0.05 MES, 0.15 M NaCl, 0.05 M sucrose; pH 6.5 when reconstituted with 0.2 ml of water **Storage:** Upon receipt store at 4ºC. Product is shipped on ice.

Introduction

The Thermo Scientific Imject Bovine Serum Albumin (BSA) and Mariculture Keyhole Limpet Hemocyanin (mcKLH) can be used for conjugating haptens to these carrier proteins to elicit an immune response and antibody production against the hapten. BSA conjugated to haptens is also used as an irrelevant carrier 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.

The MES buffer is an ideal buffer formulation for EDC conjugation. EDC is a crosslinker that reacts with carboxyl and amine groups to form stable amide bonds. Because most peptides contain both exposed lysines and carboxyl groups, EDC-mediated immunogen formation is the simplest method for most hapten-carrier protein conjugations.

Procedure for Hapten-Carrier Conjugation using EDC

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.

A. Additional Materials Required

- Hapten, 2mg
- EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride), 10mg (Product No. 77149)
- Imject EDC Conjugation Buffer (Product No. 77162), contains 0.1M MES, 0.9M NaCl, 0.02% NaN_3; pH 4.7
- Thermo Scientific Dextran Desalting Columns, 5K MWCO, (Product No. 43230)
- Imject Purification Buffer Salts (Product No. 77159), when reconstituted this buffer contains 0.083M sodium phosphate, 0.9M NaCl and sorbitol (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. Conjugation Procedure

1. Reconstitute one vial of BSA or mcKLH by adding 200µL 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. Dissolve up to 2mg of the hapten in 500µL of Imject EDC Conjugation Buffer.

   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.

3. Add the 500µL of peptide solution to the 200µL carrier protein solution.

4. For mcKLH, dissolve one vial of EDC (10mg) in 1mL of ultrapure water and immediately add 50µL of this solution to the mcKLH-peptide solution.

   For BSA, add the carrier-peptide solution to one vial of EDC (10mg) and dissolve by gentle mixing.

5. React for 2 hours at room temperature. Purify conjugate by desalting or dialysis to remove non-reacted crosslinker and sodium azide.

Additional Notes:

- Use either desalting or dialysis to remove sodium azide and excess crosslinker. 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. 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.
- PBS may be used for conjugate purification. If the conjugate will be frozen, use the Purification Buffer Salts for desalting to preserve the conjugate during freeze-thaw cycles.
- If a precipitate has formed during conjugation, centrifuge the material, collect the supernatant and save the precipitate. Apply only the supernatant to the desalting column. Combine the desalted and pooled fractions to the precipitate.
- To purify antibodies specific to the peptide, immobilize peptide through the same functional group used to prepare the immunogen. The Thermo Scientific CarboxyLink Kit (Product No. 44899) contains an amine-containing resin that will couple peptides via carboxyl groups using EDC. The peptide affinity column can 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.

Information Available from the Web

- Tech Tip #18: Block amino groups to prevent polymer formation in peptide-carrier protein conjugations
- Tech Tip #7: Remove air bubbles from columns to restore flow rate
- Tech Tip #43: Protein stability and storage

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.