

Imject[®] BSA, OVA and mcKLH

In Phosphate-based Buffers

77110 77120 77600

0117.3

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

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

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

Introduction

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 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 Imject Maleimide Conjugation Buffer (Product No. 77164), which contains 83 mM sodium phosphate, 0.1 M EDTA, 0.9 M NaCl, 0.02% sodium azide, pH 7.2, with a proprietary stabilizer
- Desalting column (e.g., Pierce[®] Polyacrylamide Desalting Columns, Product No. 43240) or dialysis cassettes (e.g., Slide-A-Lyzer[®] Dialysis Cassettes, Product No. 66810)
- Sulfhydryl-containing hapten, 20 mg
- Imject Purification Buffer Salts (Product No. 77159), upon reconstitution this buffer contains 0.083 M sodium phosphate, 0.9 M 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 2 ml of ultrapure water to make a 10 mg/ml solution.

Note: mK₂LH forms a suspension that typically appears translucent to whitish blue. Do not vortex or heat the suspension, which will cause the mK₂LH to precipitate.

2. Immediately before use prepare a ~10 mM solution of Sulfo-SMCC in ultrapure water (5 mg/ml). Add the appropriate volume of Sulfo-SMCC to the carrier protein as follows: 2 ml for mK₂LH; 3 ml for BSA; 4 ml 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 20 mg of the sulfhydryl-containing hapten in 5 ml 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 SulfoLink[®] Coupling Gel (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.

Slide-A-Lyzer[®] Dialysis Cassette Technology is protected by U.S. Patent # 5,503,741 and other patent pending.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

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

© 2007 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.