Imject® cBSA Immune Modulator

77150  77165

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
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<tbody>
<tr>
<td>77150</td>
<td>Imject cBSA Immune Modulator (in PBS), 10mg, supplied lyophilized; when reconstituted with 1mL of ultrapure water, buffer contains 0.1M sodium phosphate, 0.15M NaCl; pH 7.2 with proprietary stabilizer</td>
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<tr>
<td>77165</td>
<td>Imject cBSA Immune Modulator (in MES buffer), 2mg, supplied lyophilized; when reconstituted with 0.2mL of ultrapure water, buffer contains 0.1M MES (2-[N-morpholino]-ethanesulfonic acid), 0.15M NaCl; pH 4.7 with proprietary stabilizer</td>
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Storage: Upon receipt store product at 4°C. Product is shipped at ambient temperature.

Introduction

Thermo Scientific Imject Cationized BSA (cBSA Immune Modulator) is bovine serum albumin that has been modified by substituting anionic carboxyl groups with cationic aminoethyl-amide groups. When used as an immunogen, this cationized BSA (cBSA) stimulates a much higher antibody response than the native form of BSA. In vivo, antibody response is not only increased but remains elevated for an extended time period. In vitro, much less cBSA than native BSA is required to produce the same degree of T cell proliferation. The underlying mechanism is related to the form of immunogen that is recognized by the cells regulating the response. Because of its net positive charge (pI >11), cBSA has a greater affinity for the negatively charged cell surface membrane of the antigen-presenting cell. Rather than pinocytosis, internalization of cBSA occurs by receptor-mediated endocytosis, an adsorptive mechanism that results in more efficient uptake and processing of the immunogen.

Imject Immune Modulator is effective with both haptens and proteins for eliciting an enhanced immunological response toward the coupled molecule, which eliminates the need to develop protocols for each antigen system. This carrier will produce an anti-peptide response greater than a traditional carrier and is effective for enhancing the antibody response to proteins with low isoelectric points.

Important Product Information

- These protocols are designed to yield effective immunogens for a variety of haptens, but are 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.

- 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. When using DMSO in the conjugation, add DMSO to the buffer used 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 when purifying the conjugate. Combine the purified conjugate to the precipitate.

- PBS may be used for the final conjugate purification. If the conjugate will be frozen, use the Thermo Scientific Imject Purification Buffer Salts (Product No. 77159) for purification, which will preserve the product during freeze-thaw cycles.

- To purify the 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 Gel (Product No. 20402) contains an activated gel support that will couple peptides via sulfhydryl groups. The Thermo Scientific CarboxyLink Immobilization Kit (Product No. 44899) uses an amine-containing gel support that will couple peptides via carboxyl groups using EDC. 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.
Procedure for Maleimide Activation of cBSA and Hapten Conjugation

The Imject Immune Modulator (in PBS) is supplied in a buffer formulated for conjugation using NHS-ester chemistry. For example, the crosslinker Sulfo-SMCC contains a maleimide group that will react with free sulphydryls and a succinimidyl (NHS ester) group that will react with primary amines. By reacting the reagent to the amines on the cBSA first and then to the peptide containing a terminal cysteine, all peptide molecules will be coupled with the same orientation.

A. Additional Materials Required

- Sulfo-SMCC (sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) (Product No. 22322). Other cross-linkers, such as Sulfo-EMCS (Product No. 22307) or Sulfo-GMBS (Product No. 22324) also may be used.
- Imject Maleimide Conjugation Buffer (Product No. 77164), 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., Polyacrylamide Desalting Columns, Product No. 43240) for removing non-reacted cross-linker and EDTA. Either desalting or dialysis (Thermo Scientific Slide-A-Lyzer Dialysis Cassettes, Product No. 66810) may be used in the final purification step.
- Sulphydryl-containing hapten, 10mg

Note: If the conjugate is to be used for injection within one week, use PBS for the final purification step. If the conjugate will be frozen, use the Purification Buffer Salts for purification, which will preserve the product during freeze-thaw cycles.

B. Maleimide Activation and Conjugation Procedure

1. Reconstitute Imject Immune Modulator (in PBS) by adding 1mL of ultrapure water to make a 10mg/mL solution.
2. Immediately before use, prepare a ~10mM solution of Sulfo-SMCC in ultrapure water (5mg/mL).
3. Add 1.5mL of Sulfo-SMCC to the carrier protein and incubate for 60 minutes at room temperature or 30 minutes at 37°C with periodic gentle mixing.
4. Remove excess crosslinker using a desalting column equilibrated with Imject Maleimide Conjugation Buffer.
5. Dissolve up to 10mg of the sulphydryl-containing peptide in 2.5mL of Conjugation Buffer.
6. Immediately mix the peptide and activated cBSA and react for 2 hours at room temperature.
7. Purify the conjugate by desalting or dialysis to remove EDTA and sodium azide.

Procedure for Hapten-Carrier Conjugation using EDC

Imject Immune Modulator (in MES buffer) is supplied in a buffer formulated for EDC conjugation chemistry. EDC is a cross-linker that reacts with carboxyl and amine groups to form stable amide bonds. Because carboxyl groups on cBSA have been modified, conjugations are site-specific to cBSA amino groups and peptide carboxyl groups.

A. Additional Materials Required

- Hapten or protein, 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% NaN₃; pH 4.7
- Desalting column (e.g., Dextran Desalting Columns, 5K MWCO, Product No. 43230) or dialysis cassettes (Slide-A-Lyzer® Dialysis Cassettes, Product No. 66382)
- Imject Purification Buffer Salts (Product No. 77159), when reconstituted, this buffer contains 0.083M sodium phosphate, 0.9M NaCl and a proprietary stabilizer (see note below)

Note: If the conjugate is to be used for injection within one week, use PBS for desalting. If the conjugate will be frozen, use the Purification Buffer Salts for desalting, which will preserve the product during freeze-thaw cycles.
B. EDC Conjugation Procedure

1. Reconstitute Imject Immune Modulator (in MES buffer) by adding 200µL of ultrapure water to make a 10mg/mL solution.

2. Dissolve up to 2mg of the hapten in 0.5mL of Imject EDC Conjugation Buffer and add it to the cBSA solution.
   
   Note: If the hapten to be coupled is already in a solution free of amines or carboxylic acids and is at pH 4.7-7.2, it may be added directly to the cBSA solution.

3. Add the peptide/carrier solution to one vial of EDC (10mg) and dissolve by gentle mixing. React for 2 hours at room temperature.

4. Purify conjugate by desalting or dialysis to remove non-reacted crosslinker and sodium azide.

Additional Information Available on Our Website

Please visit our web site for additional information relating to this product including the following items:

- 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 #29: Degas buffers for use in affinity and gel filtration columns
- Tech Tip #43: Protein stability and storage

Related Thermo Scientific Products

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<th>Code</th>
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<tbody>
<tr>
<td>77140</td>
<td>Imject Freund's Complete Adjuvant, 5 × 10mL</td>
</tr>
<tr>
<td>77145</td>
<td>Imject Freund's Incomplete Adjuvant, 5 × 10mL</td>
</tr>
<tr>
<td>77161</td>
<td>Imject Alum, 50mL</td>
</tr>
<tr>
<td>77155</td>
<td>Imject Maleimide Activated cBSA, 10mg</td>
</tr>
<tr>
<td>77605</td>
<td>Imject Maleimide-Activated mcKLH, 10mg</td>
</tr>
<tr>
<td>77661</td>
<td>Imject Maleimide-Activated Blue Carrier Protein, 2mg</td>
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General References


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").

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Current product instructions are available at [www.thermoscientific.com/pierce](http://www.thermoscientific.com/pierce). For a faxed copy, call 800-874-3723 or contact your local distributor.

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