

Imject[®] Maleimide Activated Blue Carrier[®] Protein

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Number	Description
77661	Imject Maleimide Activated Blue Carrier Protein, 2mg
77662	Imject Maleimide Activated Blue Carrier Protein, 10 × 10mg
77664	Imject Maleimide Activated Blue Carrier Protein, 10mg Supplied Form: Lyophilized in 10mM sodium phosphate, 150mM sodium chloride, 10mM EDTA, 50mM sucrose; pH 7.2 Maleimide activation level: ≥ 400 moles of maleimide/mole of Blue Carrier Protein Storage: Upon receipt store at -20°C. Product is shipped on ice.

Introduction

The Thermo Scientific Imject Maleimide Activated Blue Carrier Protein is for conjugating a sulfhydryl-containing hapten to elicit an immune response and antibody production against the hapten. Blue Carrier Protein is a hemocyanin from the *Concholepas concholepas* mollusk with immunogenic properties similar to keyhole limpet hemocyanin. Blue Carrier Protein has a large mass ($\sim 8 \times 10^6$ Da) and has been successfully used in polyclonal and monoclonal antibody production for synthetic haptens and peptides.

Important Product Information

- Maleimides react with sulfhydryls at pH 6.5-7.5 to form stable thioether bonds. At pH values > 7.5, reactivity toward primary amines and hydrolysis of the maleimide group can occur.
- Haptens must have free sulfhydryl groups (-SH) available. Cysteine-containing peptides often oxidize in solution and form disulfide bonds, which cannot react with maleimides. Disulfide bonds can be reduced to produce free sulfhydryls. After reduction, most reducing reagents must be removed before conjugation. The Thermo Scientific Immobilized TCEP Disulfide Reducing Gel (Product No. 77712) enables peptide or protein reduction while recovering the sample in the absence of reducing agent.
- Sulfhydryls can be added to molecules using *N*-succinimidyl S-acetylthioacetate (SATA, Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101). Both reagents modify primary amines.

Procedure for Hapten Conjugation with Maleimide Activated Carrier Protein

A. Additional Materials Required

- Conjugation Buffer: Phosphate-buffered saline (PBS; 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2; Product No. 28372), Imject Maleimide Conjugation Buffer (Product No. 77164) or other buffer at pH 6.5-7.5
- Thermo Scientific Zeba Spin Desalting Columns (Product No. 89889, 89891, 89893), Desalting Column (Polyacrylamide Desalting Column, Product No. 43240) or dialysis cassettes (Thermo Scientific Slide-A-Lyzer Dialysis Cassette Kit, Product No. 66382)
- Imject Purification Buffer Salts (Product No. 77159)

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. Conjugation Procedure

1. Reconstitute the activated carrier protein by adding ultrapure water to make a 10 mg/ml solution.
Note: Blue Carrier Protein forms a suspension that typically appears translucent to whitish-blue. Do not vortex or heat the suspension, which will cause the carrier protein to precipitate.
2. Dissolve 2mg of the sulfhydryl-containing hapten in a volume of Conjugation Buffer equal to 1.0-2.5 times the volume of reconstituted carrier protein. For example, dissolve 2mg of peptide in 200-500µL of buffer for adding to 2mg of activated Blue Carrier Protein reconstituted in 200µL of water. Alternatively, if the peptide is freely soluble it can be added as a solid directly to the carrier protein solution.
Note: For haptens with limited solubility, DMSO may be used for solubilization. Use ≤ 30% DMSO in the final conjugation solution or the Blue Carrier Protein may irreversibly denature.
3. Immediately mix the peptide and activated carrier protein and incubate for 2 hours at room temperature.
4. Purify the conjugate by desalting (desalting column or dialysis) to remove EDTA and sodium azide, which should not be injected into laboratory animals.

Troubleshooting

Problem	Possible Cause	Solution
Hapten-Blue Carrier Protein conjugate precipitates	Peptide not readily soluble in aqueous buffer	Add up to 30% DMSO in the final reaction
	Incorrect amount of DMSO in reaction solution	Use ≤ 30% DMSO in the final reaction or the carrier protein may denature and precipitate
Hapten-Blue Carrier Protein conjugate precipitates in desalt column	DMSO added to reaction but not added to desalt buffer	Equilibrate desalting column with purification/desalt buffer containing DMSO at the same concentration as in the reaction buffer

Related Thermo Scientific Products

66382	Slide-A-Lyzer® Dialysis Cassettes, 10 dialysis cassettes appropriate for 0.5-3.0mL samples
77140	Imject Freund's Complete Adjuvant, 5 × 10mL
77145	Imject Freund's Incomplete Adjuvant, 5 × 10mL
77159	Imject Purification Buffer Salts, 5g
77161	Imject Alum, 50mL
77164	Imject Maleimide Conjugation Buffer, 30mL
89889	Zeba™ Spin Desalting Columns, 2mL, 5 columns
89891	Zeba Spin Desalting Columns, 5mL, 5 columns

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

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