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Block amino groups to prevent polymer formation in peptide-carrier protein conjugations

TR0018.2

Introduction

In heterobifunctional crosslinking reactions involving both amino and carboxyl (or other) groups, undesirable polymerization of proteins can be minimized by blocking available amino groups on one participating molecule with Thermo Scientific Sulfo-NHS Acetate (Product No. 26777). This document explains why this type of blocking step may be useful and describes a generalized protocol for use in peptide-carrier protein conjugations.

One common method for coupling small peptide antigens to immunogenic carrier proteins involves using EDC (Product No. 77149, 22980) to make attachments between carboxylates and primary amines. Some amount of end-to-end (C-terminal carboxyl group to N-terminal amino group) polymerization of peptide is tolerable and even desirable when preparing immunogens. However, each carrier protein molecule normally contains numerous carboxyl and amino groups, allowing polymerization of the carrier protein that competes with peptide coupling and may cause the carrier to aggregate and precipitate. Blocking amino groups on the carrier protein prevents such problems and forces the reaction to proceed between amines on the peptide (amino terminus and/or lysines) and carboxylates (glutamic and aspartic acids) on the carrier. (See Related Products section for a listing of available carrier proteins).

Alternatively, blocking carboxylates instead of amines forces the final reaction to proceed from carboxylates on the peptide to amines on the carrier protein. Carboxylate blocking is accomplished using EDC and a large molar excess of ethylenediamine (Product No. 23031); the result is cationized protein in which all carboxyl groups have been converted to primary amino groups. Cationized bovine serum albumin (cBSA) (Product No. 77165, 77150) and makes an excellent immunogen.

Blocking amines with Sulfo-NHS Acetate results in amino groups being capped with an acyl group; carboxyl groups are unaffected. The extent of amino group blocking can be assessed by measuring the number of amino groups before and after blocking using 2,4,6-Trinitrobenzene Sulfonic Acid (TNBSA, Product No. 28997).

Materials Required

- Protein (molecule to be modified): This method assumes use of a moderately-sized (30-200 kDa), aqueous soluble protein that contains multiple amino and carboxyl groups. **Note:** Amine blocking method is not recommended for use with the keyhole limpet hemocyanin (KLH); this carrier protein has only limited solubility, and the additional steps of handling required to block amines will cause precipitation before the final conjugation reaction can be performed.
- Reaction Buffer: 0.1 M sodium carbonate ($NaHCO_3$) buffer, pH 8.5. Other amine-free buffers may be used as alternatives, including phosphate buffered saline (PBS) and HEPES, pH 7.5-8.
- Sulfo-NHS Acetate (Product No. 26777)
- Desalting Column (Product No. 43230) or Dialysis Unit (e.g., Product No. 66385)

Procedure for Blocking Amines Using Sulfo-NHS Acetate

1. Dissolve or desalt 2.5 mg Protein into 0.5 ml Reaction Buffer.
2. Add a 25-molar excess of Sulfo-NHS Acetate to amines to the Protein solution. If the exact number of amines present is unknown, adding an equal mass amount of Sulfo-NHS Acetate to Protein will provide a large excess of reagent.
3. Mix and incubate 1 hour at room temperature.
4. Purify the blocked protein by gel filtration (Desalting Column), dialysis, or ultrafiltration. (Exchange into a buffer compatible with the downstream application; for direct use in EDC reactions, exchange into MES buffer, Product No. 28390.)

Related Thermo Scientific Products

26777	**Sulfo-NHS Acetate**, 100 mg
28997	**TNBSA**, 5% (w/v) solution of 2,4,6-Trinitrobenzene Sulfonic Acid in methanol, 100 ml
28390	**BupH™ MES Buffered Saline Packs**, 10 packs, each yields 500 ml of 0.1 M 2-[morpholino]-ethanesulfonic acid, 0.9% NaCl, pH 4.7 when dissolved in 500 ml deionized water (5 liters total)
77149, 22980	**EDC (carbodiimide)**, 10 mg and 5 g, respectively
23031	**Ethylenediamine Dihydrochloride**, 10 g
77165	**Imject® Cationized BSA (in MES buffer)**, 2 mg
77150	**Imject Cationized BSA (in PBS)**, 10 mg
77171	**Imject Bovine Serum Albumin (in MES buffer)**, 2 mg
77110	**Imject Bovine Serum Albumin (in PBS)** , 5 x 20 mg
77109	**Imject Ovalbumin (in MES buffer)**, 2 mg
77120	**Imject Ovalbumin (in PBS)**, 5 x 20 mg

More information is available from the our web site on these and other products, including carrier protein kits and maleimide-activated proteins for peptide conjugation through terminal cysteines (sulfhydryl groups).

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.

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