INSTRUCTIONS

Pierce Premium Grade Sulfo-NHS

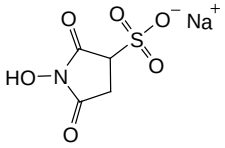
PG82071 PG82072

2535.0

Number	Description
PG82071	**Premium Grade Sulfo-NHS** (*N*-hydroxysulfosuccinimide), 500mg
PG82072	**Premium Grade Sulfo-NHS**, 10g

Molecular Weight : 217.14

CAS # 106627-54-7



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

Introduction

Thermo Scientific™ Pierce™ Premium Grade Reagents are high-quality formulations of selected chemical modification reagents, specially characterized for applications where product integrity and risk minimization are critical. Compared to standard grade equivalents, Pierce Premium Grade Reagents provide more clearly defined quality and product support by including: (a) increased analytical testing and product characterization, (b) greater batch-specific information and quality assurance review, (c) extensive lot sample retention, and (d) change control notification.

Thermo Scientific™ Pierce™ Premium Grade Sulfo-NHS is used to prepare amine-reactive esters of carboxylate groups for chemical labeling, crosslinking and solid-phase immobilization applications. Carboxylates (-COOH) may be reacted with Sulfo-NHS in the presence of a carbodiimide such as Thermo Scientific™ Pierce™ Premium Grade EDC (Product No. PG82079, PG82073 and PG82074), resulting in a semi-stable Sulfo-NHS ester, which may then be reacted with primary amines ($-NH_2$) to form amide crosslinks (Figure 1). Although Sulfo-NHS is not required for carbodiimide reactions, its use greatly enhances coupling efficiency. Furthermore, Sulfo-NHS makes it possible to perform a two-step reaction.

Sulfo-NHS is soluble in aqueous and organic solvents. Activation with Sulfo-NHS preserves or increases water-solubility of the modified molecule, by virtue of the charged sulfonate group. Although prepared Sulfo-NHS esters are sufficiently stable to process in a two-step reaction scheme, they will hydrolyze within hours or minutes, depending on water content and pH of the reaction solution (NHS esters have a half-life of 4-5 hours at pH 7, 1 hour at pH 8 and only 10 minutes at pH 8.6.).[1-3]

The activation reaction with EDC and Sulfo-NHS is most efficient at pH 4.5-7.2, and EDC reactions are often performed in MES buffer (Thermo Scientific, Product No. 28390) at pH 4.7-6.0. Reaction of Sulfo-NHS-activated molecules with primary amines is most efficient at pH 7-8, and Sulfo-NHS-ester reactions are usually performed in phosphate-buffered saline (PBS) at pH 7.2-7.5. For best results in two-step reactions, perform the first reaction in MES buffer (or other non-amine, non-carboxylate buffer) at pH 5-6, then raise the pH to 7.2-7.5 with phosphate buffer (or other non-amine buffer) immediately before reaction to the amine-containing molecule.[4] EDC reactions can be quenched with 2-mercaptoethanol (2-ME), or the excess reagent can simply be removed (as well as the reaction pH adjusted) by buffer-exchange with a desalting column (see Related Thermo Scientific Products).

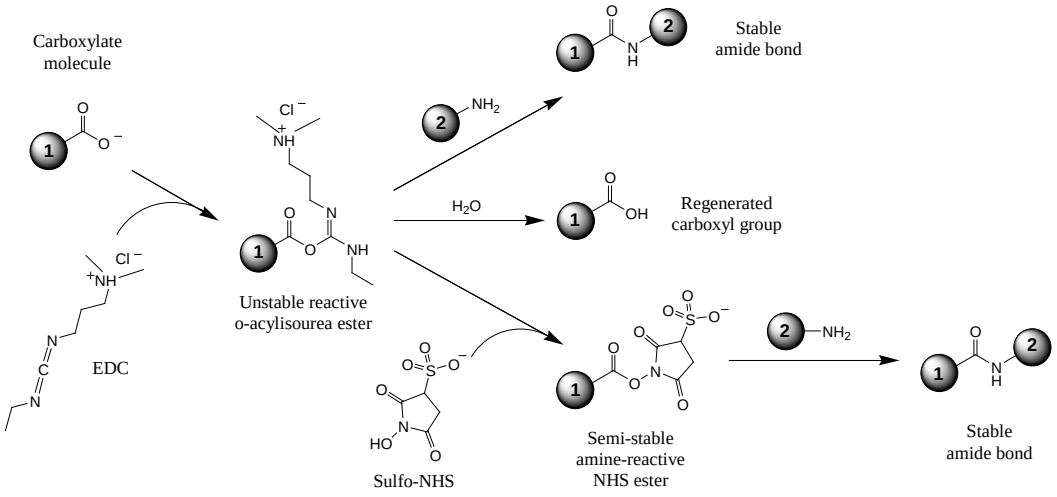


Figure 1. Reactions involving EDC, including activation as an NHS ester.

Procedure for EDC/Sulfo-NHS Crosslinking of Carboxylates with Primary Amines

A. Additional Materials Required

- Activation Buffer: 0.1M MES (2-[morpholino]ethanesulfonic acid), 0.5M NaCl, pH 6.0. Alternatively, use Thermo Scientific™ BupH™ MES Buffered Saline (Product No. 28390)
- Phosphate-buffered Saline (PBS): 0.1M sodium phosphate, 0.15M NaCl, pH 7.2-7.5 (e.g., Thermo Scientific, Product No. 28372)
- Protein #1: Prepare 1mL of Protein #1 in activation buffer at ~10mg/mL
- Protein #2: Lyophilized or dissolved at 1-10mg/mL in PBS or other amine-free buffer, pH 7-8
- Pierce Premium Grade EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide) (Product No. PG82079, PG82073, PG82074) – for best results, use a 10-fold molar excess of EDC (MW = 191.7) to Protein #1
- (Optional) 2-Mercaptoethanol (e.g., Thermo Scientific, Product No. 35600) for quenching EDC activation reaction
- (Optional) Desalting column of appropriate size for the volume of final activation reaction (e.g., Thermo Scientific™ Zeba™ Spin Desalting Columns). If intending to use this method for clean-up and buffer exchange of the activation reaction, be sure to equilibrate the desalting column so that it is ready for use when needed in Section C.
- (Optional) Hydroxylamine (e.g., Thermo Scientific, Product No. 26103) for quenching the amine reaction

B. Sulfo-NHS-ester Activation (for larger-scale activations, scale accordingly)

1. Add 0.4mg of Pierce Premium Grade EDC (final concentration 2mM) directly to 1mL of Protein #1, which, based on a 50kDa protein, results in a 10-fold molar excess of EDC to Protein #1.
2. Add 1.1mg of Pierce Premium Grade Sulfo-NHS to the reaction (final concentration 5mM).
3. Mix reaction components well and react for 15 minutes at room temperature.
4. (Optional): Add 1.4µL of 2-mercaptoethanol (final concentration of 20mM) to inactivate the EDC.
5. (Optional): Separate activated Protein #1 from excess EDC, EDC by-products, Sulfo-NHS and, if used, 2-mercaptoethanol using an appropriate size desalting column that has been equilibrated with PBS. Follow desalting column instructions and recover the fraction containing the activated protein. If using absorbance at 280nm to identify fractions containing protein, be aware that Sulfo-NHS absorbs strongly at 260-280nm.

C. Amine Reaction

1. If Step B.5 was not performed (i.e., buffer not exchanged using a desalting column), then increase buffer pH above 7.0 using concentrated PBS or other non-amine buffer such as sodium bicarbonate.
2. Add Protein #2 to the solution containing activated Protein #1.
3. Mix the solution well and then allow reaction to proceed for 2 hours at room temperature.
4. (Optional): Quench reaction by adding hydroxylamine to a final concentration of 10mM. The excess hydroxylamine reacts with all Sulfo-NHS esters remaining on the surface of Protein #1, resulting in conversion of the original carboxyl groups to a hydroxamic acid. Alternative quenching reagents include 20-50mM Tris, lysine, glycine and ethanolamine. Addition of base to raise the pH > 8 will promote hydrolysis of the Sulfo-NHS esters, thereby regenerating the original carboxyl groups.

Related Thermo Scientific Products

PG82079	**Pierce Premium Grade EDC** (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide), 1g
PG82073	**Pierce Premium Grade EDC,** 25g
PG82074	**Pierce Premium Grade EDC,** 500g
77149	**Pierce EDC,** 10mg
22980	**Pierce EDC,** 5g
22981	**Pierce EDC,** 25g
24510	**Pierce Sulfo-NHS** (N-hydroxysulfosuccinimide), 500mg
24525	**Pierce Sulfo-NHS,** 5g
24520	**Pierce Sulfo-NHS, No-Weigh™ Format**, 8 × 2mg microtubes
28390	**BupH™ MES Buffered Saline,** 10 packs, each pack results in 0.1M MES, 0.9% NaCl, pH 4.7 when dissolved in 500mL water
28372	**BupH Phosphate Buffered Saline Packs,** 40 packs, each pack results in 0.1M sodium phosphate, 0.15M NaCl, pH 7.2 when dissolved in 500mL water
89891	**Zeba Spin Desalting Columns, 7K MWCO**, 5mL, 5/pkg
89892	**Zeba Spin Desalting Columns, 7K MWCO,** 5mL, 25/pkg
89893	**Zeba Spin Desalting Columns, 7K MWCO,** 10mL, 5/pkg
89894	**Zeba Spin Desalting Columns, 7K MWCO,** 10mL, 25/pkg

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