INSTRUCTIONS

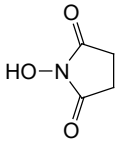
Thermo SCIENTIFIC

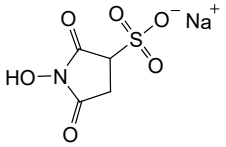
NHS and Sulfo-NHS

MAN0011309
Rev. C.0
Pub. Part No. 2160650

24500 24510 A39269 24525

Number	Description
24500	**NHS** (*N*-hydroxysuccinimide), 25g Molecular Weight: 115.10 CAS # 6066-82-6
24510	**Sulfo-NHS** (*N*-hydroxysulfosuccinimide), 500mg
A39269	**Sulfo-NHS, No-Weigh™ Format,** 10 × 2mg
24525	**Sulfo-NHS**, 5g Molecular Weight : 217.13 CAS # 106627-54-7





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

Note: Product labels have been provided for your convenience. Please label the vials using one of the labels provided in the Al foil pouch to avoid any confusion as you work with this No-Weigh reagent.

Introduction

The Thermo Scientific™ NHS and Sulfo-NHS are used to prepare amine-reactive esters of carboxylate groups for chemical labeling, crosslinking and solid-phase immobilization applications. Carboxylates (-COOH) may be reacted to NHS or Sulfo-NHS in the presence of a carbodiimide such as EDC (Product No. 22980), resulting in a semi-stable NHS or Sulfo-NHS ester, which may then be reacted with primary amines ($-NH_2$) to form amide crosslinks (Figure 1). Although NHS or Sulfo-NHS is not required for carbodiimide reactions, their use greatly enhances coupling efficiency. Furthermore, using NHS or Sulfo-NHS makes it possible to perform a two-step reaction.

Both NHS and Sulfo-NHS are soluble in aqueous and organic solvents. Activation with NHS, however, decreases water-solubility of the modified carboxylate molecule, while activation with Sulfo-NHS preserves or increases water-solubility of the modified molecule, by virtue of the charged sulfonate group. Although prepared NHS or Sulfo-NHS esters are sufficiently stable to process in a two-step reaction scheme, both groups 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] Procedures for extraction and drying can be developed to prepare stable NHS-activated molecules, but best results are obtained when NHS-activated molecules are used promptly for reaction to the amine-containing targets.

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 (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).

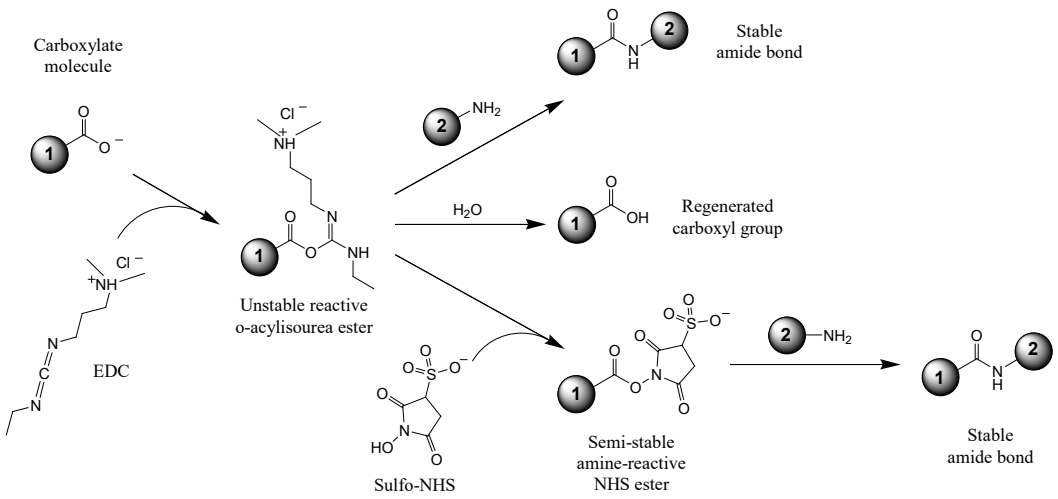


Figure 1. Reactions involving EDC, including activation as an NHS ester.

Procedure for EDC/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., 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
- EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide) (Product No. 22980) – for best results, use a 10-fold molar excess of EDC (MW = 191.7) to Protein #1
- (Optional) 2-Mercaptoethanol (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 (Product No. 26103) for quenching the amine reaction

B. NHS-ester Activation

- No-Weigh Format Handling: Immediately before use, uncap the vial and add water and mix by pipetting up and down. Alternatively, the solution can be vortexed for a few seconds to ensure a homogeneous solution. Store the unused vials in the foil pouch provided. **The maximum useable volume of the vial is 800µL.**

1. Add 0.4mg of 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 either 0.6mg of NHS or 1.1mg of Sulfo-NHS to the reaction (final concentration 5mM). If using the No-Weigh Format of Sulfo-NHS, add 40µL of ultrapure water or Activation Buffer to an individual vial, which yields 230mM; then add 22µL of the dissolved reagent to the 1mL 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-byproducts, 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 NHS and Sulfo-NHS absorb 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 to all 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 NHS esters, thereby regenerating the original carboxyl groups.

Related Thermo Scientific Products

22980	**EDC** (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide), 5g
A35391	**EDC** (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide), 10 × 1mg
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

Cited References

1. Lomant, A.J. and Fairbanks, G. (1976). Chemical probes of extended biological structures: synthesis and properties of the cleavable cross-linking reagent [^{35}S] dithiobis(succinimidyl propionate). *J Mol Biol* **104:**243-61.
2. Staros, J.V., *et al.* (1986). Enhancement by *N*-hydroxysulfosuccinimide of water-soluble carbodiimide-mediated coupling reactions. *Anal Biochem* **156:**220-2.
3. Cuatrecaseas, P. and Parikh, I. (1972). Adsorbents for affinity chromatography. Use of *N*-hydroxysulfosuccinimide esters of agarose. Biochemistry **11:**291-9.
4. Grabarek, Z. and Gergely, J. (1990). Zero-length crosslinking procedure with the use of active esters. *Anal Biochem* **185:**131-5.

Product References

Boels, K., *et al.* (2001). The neuropeptide head activator induces activation and translocation of the growth-factor-regulated Ca^{2+}-permeable channel GRC. *J Cell Sci* **114:**3599-606.

Byun, C.H., *et al.* (2001). Identification of the peptides that inhibit the function of human monoclonal thyroid-stimulating antibodies from phage-displayed peptide library. *J Clin Endocrinol Metab* **86:**3311-18.

Epstein, *A. L.* (2003). Identification of a protein fragment of interleukin 2 responsible for vasopermeability. *J Natl Cancer Inst* **95:** 741-9.

Nogami, K., *et al.* (2004). Mechanisms of interactions of Factor X and Factor Xa with the acidic region in the Factor VIII A1 domain. *J Biol Chem* **279:**3104-13.

Nyman, T., *et al.* (2002). A cross-linked profilin-actin heterodimer interferes with elongation at the fast-growing end of F-actin. *J Biol Chem* **277:**15828-33.

Remmert, K., *et al.* (2004). CARMIL is a bona fide capping protein interactant. *J Biol Chem* **279:** 3068-77.

Romaniouk, A.V., *et al.* (2004). Synthesis of a novel photoaffinity derivative of 1-deoxynojirimycin for active site-directed labeling of glucosidase I. *Glycobiology* **14:**301-10.

Van Troys, M., *et al.* (2004). TetraThymosinbeta is required for actin dynamics in *Caenorhabditis elegans* and acts via functionally different actin-binding repeats. *Mol Biol Cell* **15:**4735-48.

Witt, S., *et al.* (2004). Comparative biochemical analysis suggests that vinculin and metavinculin cooperate in muscular adhesion sites. *J Biol Chem* **279:**31533-43.

Products are 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"). 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 Buyer. Any model or sample furnished to Buyer is merely illustrative of the general type and quality of goods and does not represent that any Product will conform to such model or sample.
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 REPAIR, REPLACEMENT OF OR REFUND FOR THE NON-CONFORMING PRODUCT(S) AT SELLER'S SOLE OPTION. THERE IS NO OBLIGATION TO REPAIR, REPLACE OR REFUND FOR 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.

Unless otherwise expressly stated on the Product or in the documentation accompanying the Product, the Product is intended for research only and is not to be used for any other purpose, including without limitation, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses, or any type of consumption by or application to humans or animals.

Current product instructions are available at thermofisher.com. For a faxed copy, call 800-874-3723 or contact your local distributor.