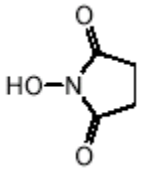
FT-UP54422

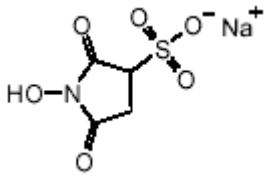
NHS / sulfo-NHS

Product Information

Catalog #: UP04594A, 500mg UP04594B, 5g
Name: **NHS (N-hydroxysuccinimide)**
C4H5NO3
MW : 115.1
CAS [6066-82-6]



Catalog #: UP54422A, 500mg
Name: **Sulfo-NHS (N-Hydroxysulfosuccinimide)**
C4H4NO6SNa
MW : 217.14
CAS [106627-54-7]



Storage: +4°C

Applications:
protein modification: conversion of carboxyl groups to amine-reactive NHS esters. Largely used as activator to mediate EDC reaction on carboxyls.

Introduction

- Improves the yield of EDC-mediated amidation/coupling
- Modify carboxyl in amines-reactive (sulfo)NHS esters
- Sulfo-NHS derivatives are water soluble, allowing crosslinking in physiologic solutions

Directions for use

Handling and Storage

NHS and SulfoNHS should be stored at +4°C. Once opened, care should be taken to avoid moisture.

Protocol (r)

This standard protocol is designed to conjugate a carboxyl-containing molecule (B) to an amine-containing molecules (A) by EDC mediated amidation with (sulfo)NHS. Each molecule tandem to conjugate may require adaptations.

1/ NHS Ester Activation of molecule B

-Add 0.4 mg EDC (~2 mM) and 0.6 mg of NHS or 1.1 mg of sulfo-NHS (~5 mM) to 1 ml of Protein #B solution.
- Mix reaction components well and react for 15 minutes at room temperature.
- (Optional): Add 1.4 µl of 2-mercaptoethanol (final concentration of 20 mM) to inactivate the EDC.
- (Optional): Separate activated Protein # B from excess EDC, EDC-byproducts, NHS (and if used 2-mercaptoethanol) using an appropriate desalting column that has been equilibrated with PBS. Recover the fraction containing the activated protein. The fractions containing protein can be identified with standard assays, we recommend a rapid colorimetric assay (Coo Assay), and not a direct spectrometric measurement at 280 nm because NHS and Sulfo-NHS absorb strongly at 260-280 nm.

2/ Reaction with amine-contain molecule A

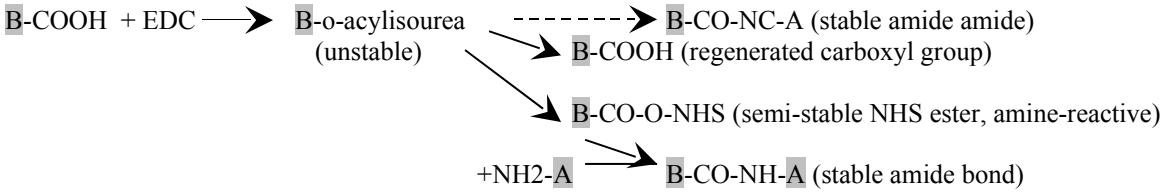
- If dessalting of B was not performed (i.e., buffer not exchanged using a desalting column), then raise buffer pH above 7 using concentrated PBS or other non-amine buffer such as sodium bicarbonate.
-Add Protein A to the solution containing activated Protein B.
-Mix the solution well and then allow reaction to proceed for 2 hours at room temperature.

-(Optional): Quench reaction by adding hydroxylamine to a final concentration of 10 mM. This method of quenching hydrolyzes any non-reacted NHS groups present on the surface of Protein B, resulting in regeneration of the original carboxyl groups. Other means of quenching involve adding 20-50 mM Tris, lysine, glycine, or ethanolamine; however, these primary amine-containing compounds will result in modified carboxyls on protein B.

Technical and Scientific Information

N-hydroxysuccinimide (NHS) reacts amines, and most interestingly with unstable reactive o-acylisourea. An important application is to mediate the reaction of carbodiimides with carboxyl groups containing molecule (B) to conjugate them with amine containing molecules (A).

- **EDC mediated amidation**
  reaction scheme:



Carbodiimide EDC (EDAC, UP52005) is a dehydrating agent used to activate carboxylate groups in (unstable) reactive o-acylisourea. Now, the formed group is unstable and short-lived in aqueous solution. As a result, EDC by itself is not particularly efficient in crosslinking because hydrolysis of the o-acylisourea, regenerating the initial carboxyl group, competes largely with the desired reaction with amines. Thus a privileged method consists to add NHS, which reacts with the o-acylisourea to yield a semi-stable amine reactive NHS-ester. The final reaction with amines is greatly favored, yielding a stable amine bond. This permits two-step crosslinking procedures, which allows the carboxyl groups eventually present on molecule A to remain unaltered.

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 the 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.)[r]. Procedures for extraction and drying may 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 usually performed in MES buffer 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 sodium 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[r]. EDC activation can be quenched by inactivation with 2-
mercaptoethanol (2-ME), or the excess reagent can simply be removed (as well as the reaction pH adjusted) by bufferexchange
with a desalting column (see Related Pierce Products). For additional discussion of EDC/NHS chemistry, including many example applications and protocols, consult the book by Hermanson (see Related Pierce Products).

- **NHS vs SulfoNHS / solubility**

N-hydroxysulfosuccinimide (Sulfo-NHS) is the sulfonated analog of NHS. Both NHS and Sulfo-NHS are soluble in aqueous and organic solvents. However, activation with NHS decreases water solubility of the modified carboxylate molecule, while activation with Sulfo-NHS (by virtue of the charged sulfonate group) preserves or increases water-solubility of the modified carboxylate molecule.

Related products

- *EDAC #* UP52005A
- *DCC #01202A*

- *Hydroxylamine #13072*
- *DSS / BS3#UP28065A/#UP54940A*
- *(sulfo)SMCC #UP3425A/#UP17412A*
- *MES Buffer GS2960*
- *PBS Buffer#UP68723A*

References

Grabarek, Z. and Gergely, J. (1990) Zero-length crosslinking procedure with the use of active esters. Anal. Biochem. 185, 131-135.
Staros, J.V., Wright, R.W. and Swingle, D.M. (1986) Enhancement by N-hydroxysulfosuccinimide of water-soluble carbodiimide-mediated coupling reactions. Anal. Biochem. 156, 220-222.
Witt, S., et al. (2004) J. Biol. Chem. 279, 31533-31543.
Biagini, R.E., et al. (2004) Clin. Diagn. Lab. Immunol. 11, 50-55.
Nyman, T., et al. (2002) J. Biol. Chem. 277, 15828-15833.

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

Rev.G03E