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A Geno Technology, Inc. (USA) brand name

Sulfo-NHS

N-Hydroxysulfosuccinimide

(Cat. # BC97)



INTRODUCTION

Sulfo-NHS is used to generate amine reactive esters from carboxylate groups and is primarily used to increase the efficiency of the carbodiimide EDC (Cat. # BC25-1, BC25-5) cross-linking. EDC reactions do not require Sulfo-NHS, however its presence greatly enhances cross-linking efficiency and allows for a two-step process. EDC



reacts with a carboxylate group to form an active ester, which is subject to rapid hydrolysis in aqueous buffers. The Sulfo-NHS increases the stability of the active intermediate and the resulting coupled product is identical to EDC reactions without sulfo-NHS.

Sulfo-NHS is water soluble and long-lived and hydrolyzes relatively slowly in water and aqueous buffer, unlike NHS.

ITEM(S) SUPPLIED (Cat. #BC97)

Description	Size
Sulfo NHS	500mg

STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store it refrigerated at 4°C. This product is stable for 1 year at 4°C.

SPECIFICATIONS

- Molecular Formula: C₄H₄NNaO₆S
- Molecular weight: 217.13
- CAS # 106627-54-7
- Water soluble

PROTOCOL: TWO STEP METHOD

- Prepare a 1-10mg/ml protein solution in 0.1M sodium phosphate, pH7.4.
 NOTE: If using KLH, prepare in 0.1M sodium phosphate, 0.9M NaCl pH7.4 to maintain the solubility of the high molecular weight protein. Sodium chloride can be added to the buffer if solubility is a concern.
- Dissolve the peptide or molecule to be coupled in the same buffer as the protein.
 For small molecules use at least a 10-fold molar excess over the protein.
- 3. Combine the protein and peptide solutions, ensuring at least a 10-fold molar excess of peptide to protein.
- 4. Add EDC to the reaction to give a final 10-fold molar excess over the protein.
- 5. Once dissolved, immediately add Sulfo-NHS to a final 5mM concentration and vortex to mix.

NOTE: If a precipitation occurs, scale back the amount of EDC and sulfo-NHS used.

- 6. React for 2 hours at room temperature.
- 7. Purify the conjugate by dialysis or gel filtration.

ALTERNATIVE PROTOCOL

The variations in the pH below increases the stability of the active ester intermediate. This allows for isolation of the active species within a reasonable time frame without significant loss of activity. β-mercaptoethanol is added to quench the unreacted EDC.

- 1. Dissolve protein to 1mg/ml in 0.05M MES, 0.5M NaCl, pH6.
- 2. Add EDC to give a final concentration of 2mM and then add Sulfo-NHS to give a final concentration of 5mM.
- 3. Mix and incubate at room temperature for 15 minutes.
- 4. Add β -mercaptoethanol to a final concentration of 20mM. Incubate at room temperature for 10 minutes to inactivate free EDC.
- 5. Add the quenched, activated protein to the peptide, ensuring a 10-fold molar excess of peptide. Prepare the peptide in 0.1M sodium phosphate, pH7.5.
- 6. React for 2 hours at room temperature.
- 7. Purify the conjugate by dialysis or gel filtration.

RELATED PRODUCTS

Download our Protein Labeling & Conjugation Handbook.



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