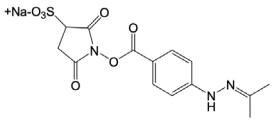
USER GUIDE

Sulfo S-HyNic Linker (Water Soluble)



$C_{14}H_{14}N_3NaO_7S$; Mol. Wt.: 391.33

vector LABORATORIES

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Cat. No. S-1011-010

Storage Desiccated: -15° to -25°C

Introduction

SoluLINK® bioconjugation technology is based on the formation of a stable covalent bond that has a UV-traceable signal to indicate the real-time formation of conjugate. This bond is a bis-aryl hydrazone formed from an aromatic hydrazine and an aromatic aldehyde. Sulfo S-HyNic (sulfosuccinimidyl 6-hydrazinonicotinate acetone hydrazone) is a water-soluble version of S-HyNic which is used to incorporate aromatic hydrazine linkers on biomolecules and surfaces. Sulfo S-HyNic is an amine-reactive linker that directly converts amino groups to HyNic groups. S-4FB (succinimidyl 4-formylbenzoate) is used to convert amino groups to aromatic aldehydes (4-formylbenzamide, or 4FB groups). Addition of a HyNic-modified biomolecule to a 4FB-modified biomolecule or surface leads directly to the formation of the conjugate (Figure 1). The conjugate bond is stable to 92°C and pH 2.0–10.0. The recommended pH for biomolecule modification is 8.0, and conjugation is 6.0. Unlike thiol-based conjugation protocols where reducing reagents are required that can compromise the activity of proteins by cleaving disulfide bonds, the HyNic-4FB conjugation couple leaves disulfide bonds intact. No oxidants, reductants or metals are required in the preparation of conjugate.

Further enhancing the many advantages of the HyNic/4FB conjugation couple is the discovery Dirksen et al. that showed aniline catalyzes the formation of this Schiff's base. This is especially effective for large biomolecule conjugations. In the case of antibody-protein conjugations the addition of 10 mM TurboLINK™ Catalyst Buffer (10X) (aniline) to the reaction converts >95% of the antibody to conjugate in ~2 hours using 1–2 mole equivalents of the second protein.

The HyNic-4FB conjugation couple is chromophoric - the conjugate bond absorbs at 354 nm and has a molar extinction coefficient of 29,000 L/(mol*cm). This allows (1) real time spectrophotometric monitoring of a conjugate reaction, (2) ability to 'visualize' the conjugate during chromatographic purification using a UV or photodiode array detector and (3) quantification of conjugation. Furthermore, the level of incorporation of HyNic groups can be quantified colorimetrically as reaction with 2-Sulfobenzaldehyde yields a chromophoric product that absorbs at 350 nm with a molar extinction coefficient of 28,500 L/(mol*cm).

Sulfo S-HyNic is a water-soluble sulfo NHS ester linker that converts amines on biomolecules and surfaces to HyNic groups. Sulfo-S-HyNic is recommended for modification of any amino surfaces such as beads and quantum dots.

Links to procedures and calculators are provided in this user guide. (Also see Application Notes).

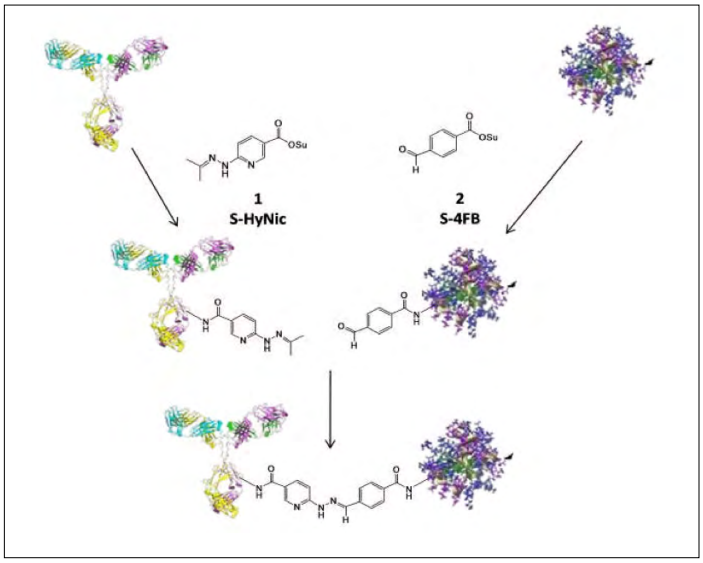

Figure 1: Schematic representation of SoluLINK bioconjugation chemistry where an antibody is modified with S-HyNic to incorporate HyNic groups and a second protein is modified with S-4FB to incorporate 4FB groups. Conjugate is formed directly by simply mixing the HyNic-modified antibody with the 4FB-modified protein.

Additional materials required

Reagents

Thermo Scientific™ Zeba™ Desalting Columns

Modification Buffer (10X)

Conjugation Buffer (10X)

Anhydrous DMF

2-Sulfobenzaldehyde

Equipment

Variable-speed bench-top microcentrifuge

Spectrophotometer or Plate Reader

1.5 ml microcentrifuge tubes

Modification Procedure

A. Desalting

1. Desalt/buffer exchange the protein into 1X Modification Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 8.0). If needed, refer to the Protein Desalting Protocol or Oligonucleotide Desalting Protocol.

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Continued from page 1.

Notes:

a) It is necessary to remove all free amine-containing contaminants, e.g., tris or glycine, from the protein before modification.

b) High-level buffering capacity, i.e. 100 mM phosphate, is necessary for successful modification.

c) For desalting proteins, Zeba Desalting Columns are recommended; for oligonucleotides, Sartorius Vivaspin diafiltration units. Refer to desalting protocol for either apparatus.

B. Determine the concentration of the desalted protein

1. Determine the concentration of the protein to be modified using a Bradford assay or BCA assay or oligonucleotide by using the Oligonucleotide Concentration Determination Protocol. Alternatively the A280 can be used if the protein extinction coefficient is known (E1%).
2. Adjust the concentration to 1.0–2.5 mg/ml in 1X Modification Buffer, pH 8.0, if necessary.

C. Prepare Sulfo S-HyNic/DMF stock solution

1. Prepare a stock solution of Sulfo-S-HyNic in anhydrous DMF or aqueous buffer by dissolving 2–4 mg of Sulfo-S-HyNic in 100 µl anhydrous DMF or in aqueous buffer.

   Note:

   a) The Sulfo-S-HyNic/DMF stock solution is stable for 2 weeks if prepared with anhydrous DMF and stored desiccated.

   b) Stock solution prepared in buffer must be used immediately.

D. Modification of a protein

1. Using Table 1 as a guide, add the required volume of Sulfo S-HyNic solution the protein solution. Refer to the Protein Modification Calculator, or Amino-Oligonucleotide Modification Calculator, if needed.

IgG Concentration (mg/ml)	Sulfo S-HyNic Mole Equivalents Added	Determined Ratio of HyNic/Protein
1.0	20	5.5
	30	8.2
4.0	15	4.7
	20	6.4
	25	7.8

Table 1: The number of HyNic groups incorporated on an antibody is dependent on the number of mole equivalents sulfo-S-HyNic added and the protein concentration. This table can be used as a general guide for modification of any protein with a succinimidyl-based modification reagent.

2. Allow the reaction to incubate at room temperature for 2.0 hours.

E. Desalting procedure

1. Desalt/buffer exchange the protein into 1X Conjugation Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 6.0). If needed, refer to the Protein Desalting Protocol or Oligonucleotide Desalting Protocol.

F. Quantifying modification level

1. The molar substitution ratio (MSR) can be determined using a colorimetric reaction outlined in Figure 2. Addition of 2-sulfobenzaldehyde to a HyNic-modified biomolecule yields a bis-aryl hydrazone that absorbs at 350 nm. Refer to the HyNic-Protein MSR Calculator as well as the protocol that is appropriate for your lab equipment: HyNic Protein MSR Instructions.

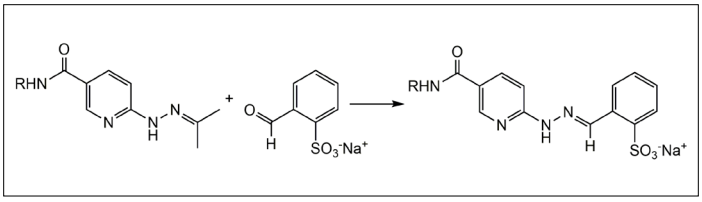

Figure 2: Colorimetric reaction used to quantify number of HyNic moieties on a biomolecule.

2. The biomolecule is now HyNic-modified and ready for conjugation to 4FB-modified biomolecules and surfaces.

Application Notes

Performing a Bradford assay

Performing a BCA protein assay

Oligonucleotide Concentration Determination Protocol

Protein Desalting Protocol

Oligonucleotide Desalting Protocol

HyNic Protein MSR Instructions

Troubleshooting Guide

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