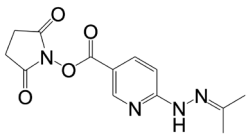
USER GUIDE

S-HyNic Linker (DMF Soluble)



$C_{13}H_{14}N_4O_4$; Mol. Wt.: 290.27

Together we breakthrough™

Cat. No. S-1002

Storage Desiccated: -15° to -25°C

Introduction

SoluLINK® bioconjugation technology is based on the formation of a stable covalent bond between an aromatic hydrazine and an aromatic aldehyde. S-HyNic (succinimidyl 6-hydrazinonicotinate acetone hydrazone) is used to incorporate aromatic hydrazine linkers on biomolecules. S-HyNic is an amine-reactive linker that directly converts amino groups (e.g., lysines) on biomolecules and surfaces 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 conjugates.

Further enhancing the many advantages of the HyNic/4FB conjugation couple is the discovery by 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 buffer) 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 and yields a chromophoric product that absorbs at 350 nm with a molar extinction coefficient of 28,500 L/(mol*cm).

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

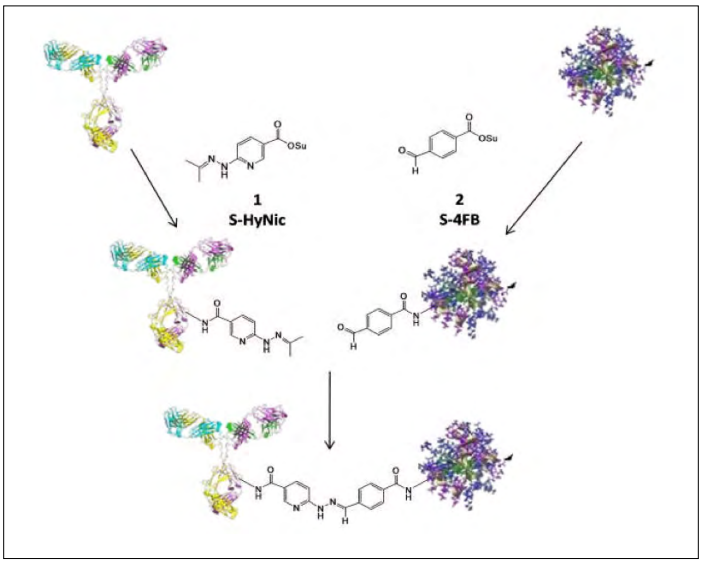


Figure 1: Schematic representation of Solulink Bioconjugation chemistry where an antibody is modified with S-HyNic to incorporate HyNic groups and a second protein is modified with S-4FB to incorporate 4FB groups. Conjugate is formed directly by simply mixing the HyNic-modified antibody with the 4FB-modified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

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.

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Continued from page 1.

Notes:

a) Buffer exchange removes all free amine-containing contaminants, e.g., tris or glycine, from the protein solution 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.

B. Determine the concentration of the desalted protein

1. Determine the concentration of the protein to be modified using a spectrophotometer and the known E1% (280 nm). Alternatively, a Bradford assay or BCA assay can be used if the protein extinction coefficient is not known.
2. Adjust the concentration to 1.0–4.0 mg/ml in 1X Modification Buffer, pH 8.0, if necessary.

C. Prepare S-HyNic/DMF stock solution

1. Prepare a stock solution of S-HyNic in anhydrous DMF (or DMSO) by dissolving 2–4 mg of S-HyNic in 100 µl anhydrous DMF.

D. Modification of a protein

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

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

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

2. Immediately pipet up and down, then gently vortex to mix.
3. 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.

F. Quantifying the molar substitution ratio (MSR)

1. The molar substitution ratio (MSR) can be determined using a colorimetric reaction as outlined in Figure 2 below. 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.

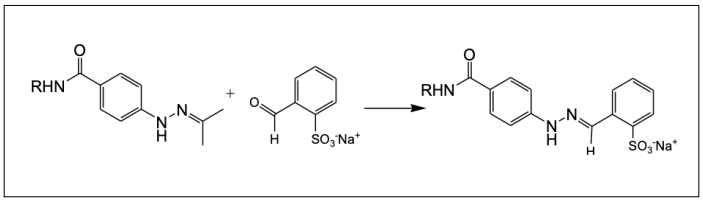

Figure 2: Colorimetric reaction used to quantify number of HyNic linkers on a biomolecule.

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

Note: HyNic-modified biomolecules should be used immediately for conjugation.

Application Notes

Performing a Bradford assay

Performing a BCA protein assay

Protein Desalting Protocol

Protein Modification Calculator

HyNic-Protein MSR Calculator

HyNic Protein MSR Instructions

Troubleshooting Guide

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