



# SZABO SCANDIC

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## Produktinformation



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siehe unsere [Liefer- und Versandbedingungen](#)

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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

### SZABO-SCANDIC HandelsgmbH

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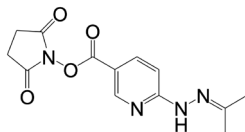
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# S-HyNic Linker (DMF Soluble)

C<sub>13</sub>H<sub>14</sub>N<sub>4</sub>O<sub>4</sub>; Mol. Wt.: 290.27



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](#)).

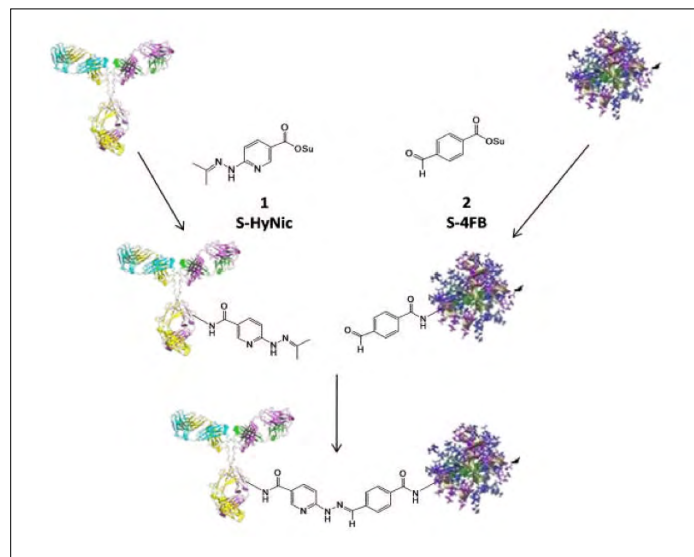


Figure 1: Schematic representation of Solulink Bioconjugation chemistry where an antibody is modified with S-HyNic to incorporate HyNic groups and a second protein is modified with S-4FB to incorporate 4FB groups. Conjugate is formed directly by simply mixing the HyNic-modified antibody with the 4FB-modified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

- 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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**Notes:**

- Buffer exchange removes all free amine-containing contaminants, e.g., tris or glycine, from the protein solution before modification.
- High-level buffering capacity, i.e. 100 mM phosphate, is necessary for successful modification.
- For desalting proteins, Zeba Desalting Columns are recommended.

**B. Determine the concentration of the desalted protein**

- 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.
- 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**

- 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**

- 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	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 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.

- Immediately pipet up and down, then gently vortex to mix.
- Allow the reaction to incubate at room temperature for 2.0 hours.

**E. Desalting procedure**

- 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)**

- 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](#).

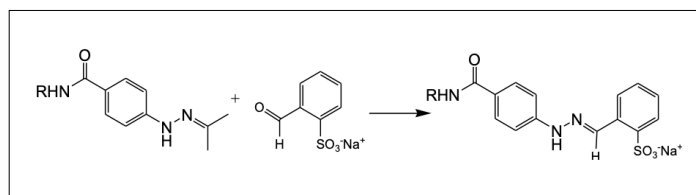


Figure 2: Colorimetric reaction used to quantify number of HyNic linkers on a biomolecule.

- 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](#)