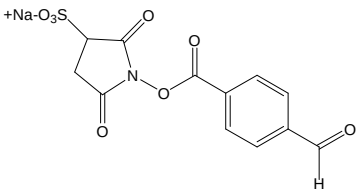
Sulfo-S-4FB

$C_{12}H_8NNaO_8S$ MW 349.25
Catalog# S-1008



Introduction to Solulink Bioconjugation Technology

Solulink's core technology is based on the formation of a covalent bond formed from an aromatic hydrazine and an aromatic aldehyde. S-HyNic **1** (succinimidyl 6-hydrazinonicotinate acetone hydrazone, SANH) is used to incorporate aromatic hydrazine linkers on biomolecules. S-HyNic is an amino-reactive reagent that directly converts amino groups on biomolecules and surfaces to HyNic groups. S-4FB **2** (succinimidyl 4-formylbenzoate, SFB) is used to convert amino groups to aromatic aldehydes (4-formylbenzamide (4FB) groups). Addition of a HyNic-modified biomolecule to a 4FB-modified biomolecule or surface directly leads to the formation of the conjugate (Figure 1). The conjugate bond is stable to 92^{O}C and pH 2.0-10.0. The recommended pH for antibody conjugation is 6.0. Unlike thiol-based conjugation protocols where reducing reagents are required that 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.

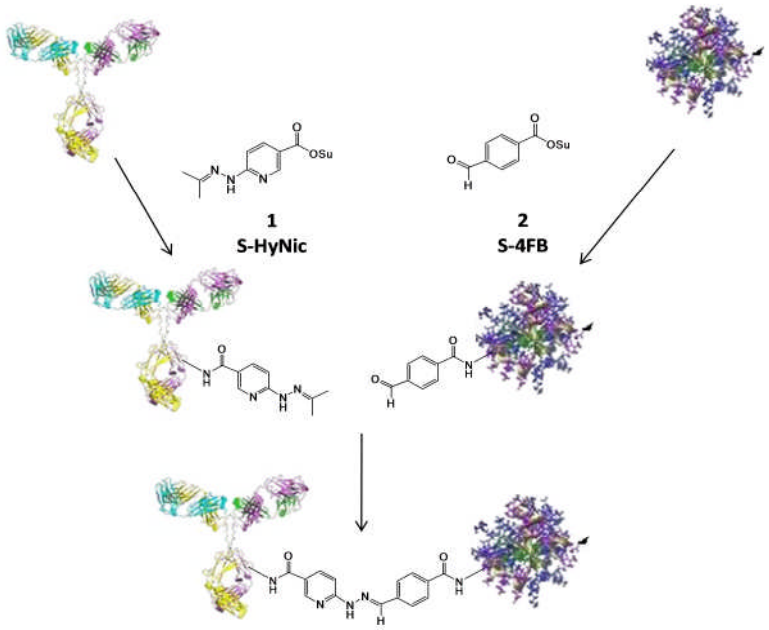


Figure 1: Schematic representation of Solulink Bioconjugation chemistry where an antibody is modified with S-HyNic to incorporate HyNic groups and a second protein is modified with S-4FB to incorporate 4FB groups. Conjugate is formed directly by simply mixing the HyNic-modified antibody with the 4FB-modified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

Further enhancing the many advantages of the HyNic/4FB conjugation couple is the discovery by Dirksen *et al.*[1] that showed that 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 (aniline) to the reaction mixture converts >95% of the antibody to conjugate in ~2 hours using 1-2 mole equivalents of second protein.

The HyNic-4FB conjugation couple is chromophoric- the conjugate bond absorbs at 354 nm and has a molar extinction coefficient of 29000. 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 4FB groups can be quantified colorimetrically as reaction with 2-hydrazinopyridine (Solulink catalog# # S-2002) yields a chromophoric product that absorbs at A354 with a molar extinction coefficient of 28,500 (Figure 2). Links to procedures and calculators to guide users through this process are given in the procedures below.

Sulfo-S-4FB is a water soluble analog of S-4FB. Sulfo-S-4FB is recommended for use to incorporate 4FB linkers on amino-surfaces and when modifying biomolecules when DMF or DMSO is deleterious to the biomolecule.

Methods

Additional Materials Required

Reagents		Equipment
Diafiltration spin columns	S-4004	Variable-speed bench-top microcentrifuge
Modification Buffer	S-4000	Spectrophotometer or Plate Reader
Conjugation Buffer	S-4002	1.5 mL microcentrifuge tubes
Anhydrous DMF	S-4001	2-Sulfobenzlaldehyde (S-2005)

Note: This protocol and all documents linked below can be downloaded from the appropriate category in the Solulink Library at http://www.solulink.com/library.

Modification Procedure

A. Desalting procedure

1. Desalt/buffer exchange the protein or oligonucleotide into 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**, if needed.

Notes:

a) It is necessary to remove all free amine-containing contaminants, *e.g.* tris, 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 Solulink recommends Pierce Zeba Desalt Spin columns (# 89882); for oligonucleotides, Sartorius Vivaspin diafiltration units (#VS0112). Refer to desalting protocol for either apparatus.

B. Determine the concentration of the protein

1. Determine the concentration of the protein to be modified using a **Bradford assay** (BioRad, #500-0006) or the **BCA assay** (ThermoScientific, #23223). Alternatively the A280 can be used if the protein extinction coefficient is known (E1%).
2. Adjust the concentration to 1-2.5 mg/mL in Modification Buffer pH 8.0, if necessary

IgG concentration (mg/mL)	Sulfo-S-4FB molar equivalents added	Determined ratio of 4FB/protein
1.0	5	2.38
	10	4.73
	15	6.20
2.5	5	3.08
	10	6.58
5.0	5	3.74
	10	6.80

Table 1: The number of 4FB groups incorporated on an antibody is dependent on the number of mole equivalents S-4FB 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.

C. Prepare a Sulfo-S-4FB/DMF stock solution

1. Prepare a stock solution of Sulfo-S-4FB in anhydrous DMF by dissolving 2-4 mg of Sulfo-S-4FB in 100 µL anhydrous DMF or buffer.

Note:

a) The Sulfo-S-4FB/DMF stock solution is stable for 2 weeks if prepared with anhydrous DMF (Solulink catalog# S-4001) and stored desiccated.
b) Stock solution prepared in buffer must be used immediately.

D. Modification of a protein or oligonucleotide

1. Using Table 1 as a guide, add the requisite volume of Sulfo-S-4FB/DMF to the protein solution; refer to the **Protein Modification With An NHS Ester Calculator** or **Amino-Oligonucleotide Modification With An NHS Ester Calculator**, if needed.
   a. Note: be sure to use the correct values for Sulfo-S-4FB in the ***Reagent Information*** section of the calculator
2. Allow reaction to incubate at room temperature for 2.0 hours.

E. Desalting procedure

1. Desalt/buffer exchange the protein or oligonucleotide into 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**, if needed.

F. Quantifying modification level

1. The molar substitution ratio (MSR) can be determined using a colorimetric reaction outlined in Figure 2. Addition of 2-hydrazinopyridine to a 4FB-modified biomolecules yields a compound that absorbs at 350 nm. Refer to the **4FB-Protein Colorimetric MSR Calculator** or the **4FB-Oligonucleotide Colorimetric MSR Calculator** as well as the protocol that is appropriate for your lab equipment: **4FB Colorimetric MSR Assay Protein Nanodrop Method Protocol**, **4FB Colorimetric MSR Assay Protein Spectrophotometer Method Protocol**, **4FB Colorimetric MSR Assay Oligonucleotide Nanodrop Method Protocol**, or **4FB Colorimetric MSR Assay Oligonucleotide Spectrophotometer Method Protocol**.

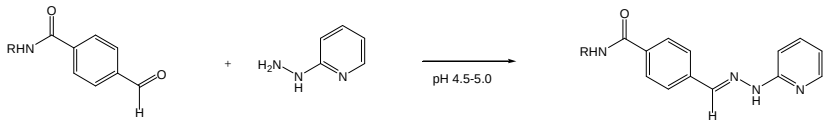


Figure 2: Colorimetric reaction used to quantify number of 4FB linkers on a biomolecule

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

Troubleshooting

Problem	Possible Cause	Solution
Protein was not modified or poorly modified.	Protein has been contaminated with amine containing compounds	Desalt the protein more thoroughly with a new Zeba Spin column or VivaSpin diafiltration apparatus
	The concentration of the protein was too low	Increase the concentration of the protein to >2.0 mg/mL
Sulfo-S-4FB was hydrolyzed	The buffer hydrolyzed the NHS ester	Modify the surface, protein or oligo with the linker immediately after dissolving.

Stability

The 4FB linkers incorporated on biomolecules are stable at 4^{O}C for >30 days.

Related Solulink Products

S-4004	VivaSpin diafiltration device	S-2002	2-hydrazinopyridine	S-2006	TurboLink Catalyst Buffer
S-1037	S-SS-4FB Linker	S-4001	Anhydrous DMF		
S-4000	Modification Buffer	S-4002	Conjugation Buffer		

References

1. Dirksen, A., et al., Nucleophilic catalysis of hydrazone formation and transimination: implications for dynamic covalent chemistry. J Am Chem Soc, 2006. 128(49): p. 15602-3.
2. Ryan C. Bailey, Gabriel A. Kwong, Caius G. Radu, Owen N. Witte, and James R. Heath, DNA-Encoded Antibody Libraries: A Unified Platform for Multiplexed Cell Sorting and Detection of Genes and Proteins, J. Amer. Chem. Soc. 2007, **129**, 1959-1967.