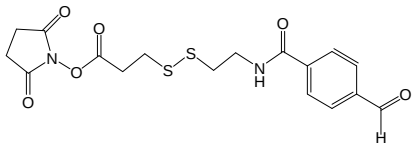
S-SS-4FB

$C_{17}H_{18}N_2O_6S_2$ MW 410.46
Catalog# S-1037



Introduction to Solulink Bioconjugation Technology

Solulink's core technology is based on the formation of a stable aromatic bond that has a UV-traceable signal to indicate the real-time formation of the conjugate. 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.

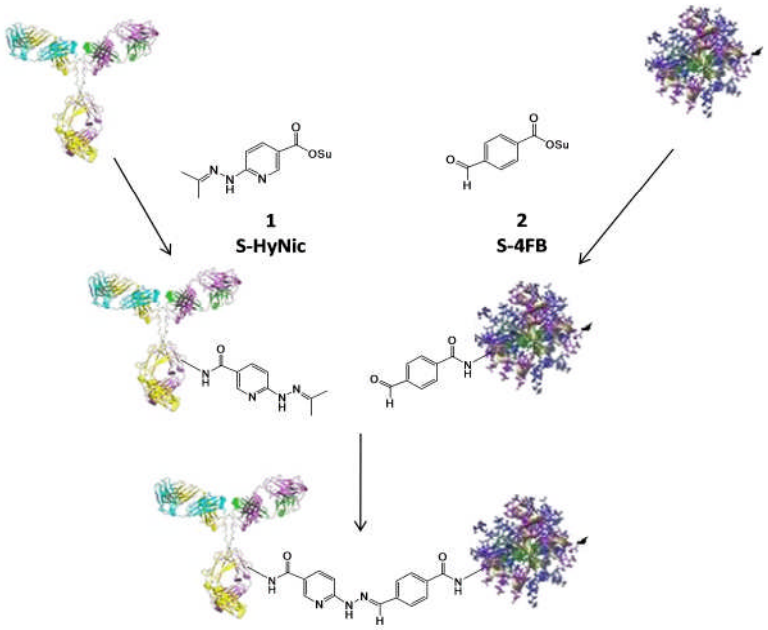


Figure 1: Schematic representation of SoluLinK Bioconjugation chemistry where an antibody is modified with S-HyNic to incorporate HyNic groups and a second protein is modified with S-4FB to incorporate 4FB groups. Conjugate is formed directly by simply mixing the HyNic-modified antibody with the 4FB-modified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

S-SS-4FB is an analog of S-4FB with a disulfide cleavable moiety in the linker if required for intracellular cleavable of the conjugate.

Methods

Additional Materials Required

Reagents		Equipment
Diafiltration spin columns	S-4004	Variable-speed bench-top microcentrifuge
Modification Buffer	S-4003	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)	S-SS-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-SS-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 S-SS-4FB/DMF stock solution

1. Prepare a stock solution of S-SS-4FB in anhydrous DMF (or DMSO).
   Note: The S-SS-4FB/DMF stock solution is stable for 2 weeks if prepared with anhydrous DMF (Solulink Cat. No. S-4001) and stored desiccated. We recommend dissolving 1 mg of S-SS-4FB in 100 µL DMF and 10 mg of S-SS-4FB in 500 µL DMF.

D. Modification of protein or oligonucleotides

1. Using Table 1 as a guide, add the requisite volume of S-SS-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 S-SS-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**.

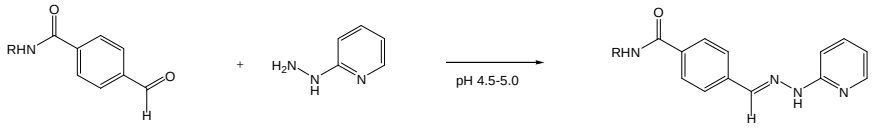


Figure 2: Colorimetric reaction used to quantify number of 4FB linkers on a biomolecule

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
S-SS-4-FB was hydrolyzed	Wet or poor quality DMF/DMSO hydrolyzed the NHS ester	Use a good quality anhydrous DMF/DMSO to solubilize S-SS-4-FB.

Stability

The SS-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-1004	S-4FB Linker	S-4001	Anhydrous DMF		
S-4003	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.