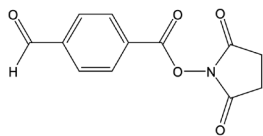


S-4FB Linker (DMF Soluble)

C₁₂H₉NO₅; Mol. Wt.: 247.20



Cat. No. S-1004

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 conjugate.

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 4FB groups can be quantified colorimetrically as reaction with 2-Hydrazinopyridine dihydrochloride and yields a chromophoric product that absorbs at 350 nm with a molar extinction coefficient of 24,500 L/(mol*cm).

S-4FB directly modifies amine groups on biomolecules and surfaces to incorporate 4FB (4-formylbenzamide) linkers. For modifying amine-containing surfaces, it is recommended to use Sulfo S-4FB Linker (Water Soluble).

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

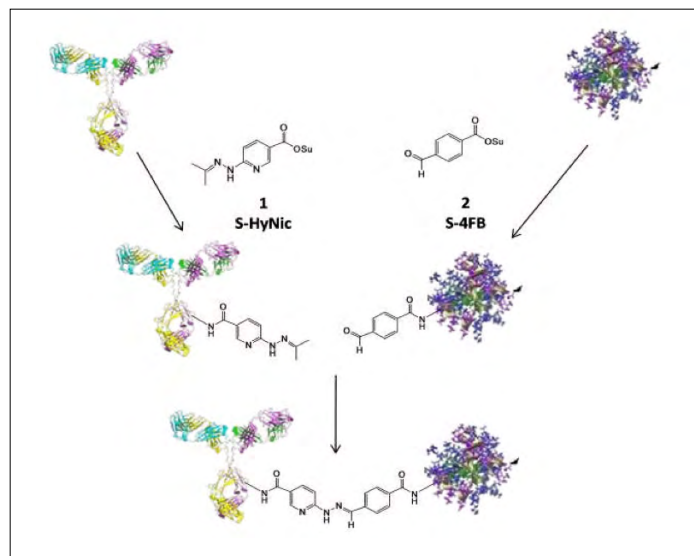


Figure 1: Schematic representation of SoluLINK bioconjugation chemistry where an antibody is modified with S-HyNic to incorporate HyNic groups and a second protein is modified with S-4FB to incorporate 4FB groups. Conjugate is formed directly by simply mixing the HyNic-modified antibody with the 4FB-modified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

Additional materials required

Reagents

Thermo Scientific™ Zeba™ Desalting Columns
Modification Buffer (10X)
Conjugation Buffer (10X)
Anhydrous DMF
2-Hydrazinopyridine dihydrochloride

Equipment

Variable-speed bench-top centrifuge
Spectrophotometer or Plate Reader
1.5 ml microcentrifuge tubes

Modification Procedure

A. Desalting

- Desalt/buffer exchange the protein or oligonucleotide 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](#).

Continued on next page.

Notes:

- a) It is necessary to remove all free amine-containing contaminants, e.g., tris or glycine, from the protein or oligo 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. For oligonucleotides, VivaSpin™ or Amicon™ diafiltration units are recommended. Refer to the desalting protocols.

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 1.0–5.0 mg/ml in 1X Modification Buffer, pH 8.0, if necessary.

C. Prepare S-4FB/DMF stock solution

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

D. Modification of a protein

- 1. Using Table 1 as a guide, add the required volume of S-4FB/DMF to the protein solution. Refer to the [Protein Modification Calculator](#) or [Amino-Oligonucleotide Modification Calculator](#), if needed.

IgG Concentration (mg/ml)	S-4FB Mole 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 a protein with an NHS ester-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 or oligonucleotide 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-hydrazinopyridine to a 4FB-modified biomolecule yields a bis-aryl hydrazone that absorbs at 350 nm. Refer to the [4FB-Protein MSR Calculator](#) or the [4FB-Oligonucleotide MSR Calculator](#) as well as the protocol that is appropriate for your lab equipment: [4FB Oligo MSR Instructions](#) or [4FB Protein MSR Instructions](#).

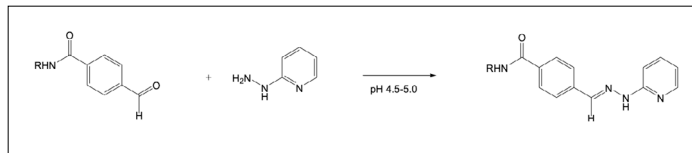


Figure 2: Colorimetric reaction used to quantify the number of 4FB linkers on a biomolecule.

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

Application Notes

- [Performing a Bradford assay](#)
- [Performing a BCA protein assay](#)
- [Protein Desalting Protocol](#)
- [Oligonucleotide Desalting Protocol](#)
- [4FB Oligo MSR Instructions](#)
- [4FB Protein MSR Instructions](#)
- [Troubleshooting Guide](#)