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Produktinformation

- Forschungsprodukte & Biochemikalien
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Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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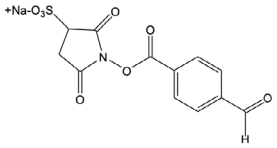
USER GUIDE

Sulfo S-4FB Linker (Water Soluble)



$C_{12}H_8NNaO_8S$; Mol. Wt.: 349.25

vector LABORATORIES

Together we breakthrough™

Cat. No. S-1008

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. Sulfo S-4FB (sulfo 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).

Sulfo S-4FB is a water-soluble analog of S-4FB. Sulfo S-4FB is recommended to incorporate 4FB linkers on amino-surfaces, and for modifying delicate biomolecules when even small amounts of DMF or DMSO is incompatible with the biomolecule.

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

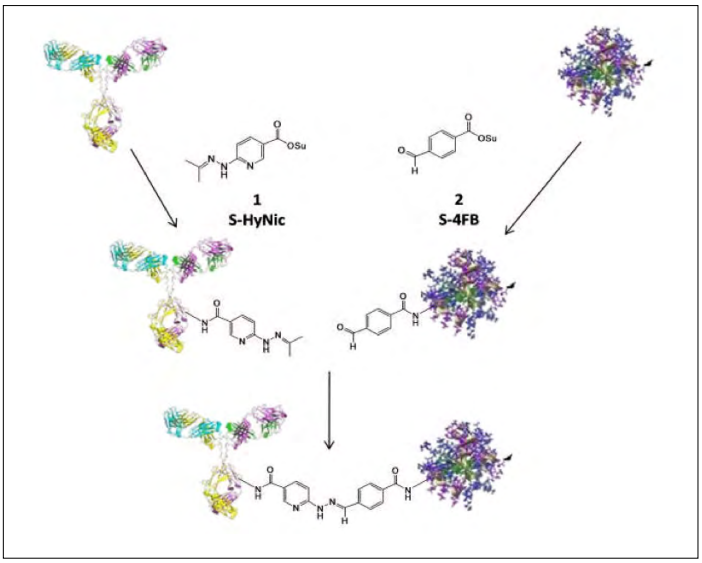


Figure 1: Schematic representation of SoluLINK bioconjugation chemistry where an antibody is modified with S-HyNic to incorporate HyNic groups and a second protein is modified with S-4FB to incorporate 4FB groups. Conjugate is formed directly by simply mixing the HyNic-modified antibody with the 4FB-modified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

1. 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.

Continued from page 1.

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 stock solution

1. Prepare a stock solution of Sulfo S-4FB in water or 1X Modification Buffer by dissolving 2–4 mg of Sulfo S-4FB in 100 µl of water or buffer. Alternatively, anhydrous DMF may be used to prepare the stock solution, if desired.

   Note: The Sulfo S-4FB stock solution prepared in water or aqueous buffer must be used immediately, as NHS esters rapidly hydrolyze.

D. Modification of a protein

1. Using Table 1 as a guide, add the required volume of Sulfo S-4FB to the protein or oligo solution. Refer to the Protein Modification Calculator or Amino-Oligonucleotide Modification Calculator, if needed.

IgG Concentration (mg/ml)	Sulfo S-4FB Mole Equivalents Added	Determined Ratio of 4FB/Protein
1.0	5 10 15	2.38 4.73 6.20
2.5	5 10	3.08 6.58
5.0	5 10	3.74 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 Protein MSR Instructions or 4FB Oligo MSR Instructions.

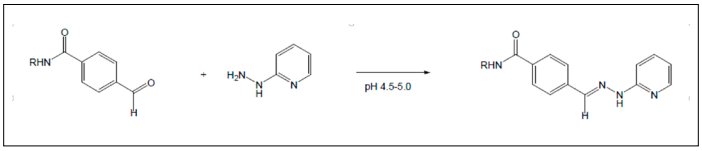


Figure 2: Colorimetric reaction used to quantify the number of 4FB linkers on a biomolecule.

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 Protein MSR Instructions

4FB Oligo MSR Instructions

Troubleshooting Guide

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