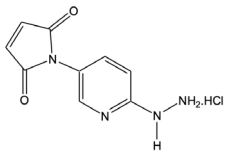
VECTOR LABORATORIES

part of Maravai LifeSciences

USER GUIDE

MHPH (Maleimide HyNic) Linker



$C_9H_8N_4O_2$.HCl; Mol. Wt.: 240.65

Cat. No.: S-1009-010

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. MHPH (Maleimide HyNic) is a thiol-reactive linker that converts thiol groups on biomolecules and surfaces to HyNic groups. **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 with MHPH is 6.5, and conjugation is 6.0. No toxic metals or oxidants 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 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).

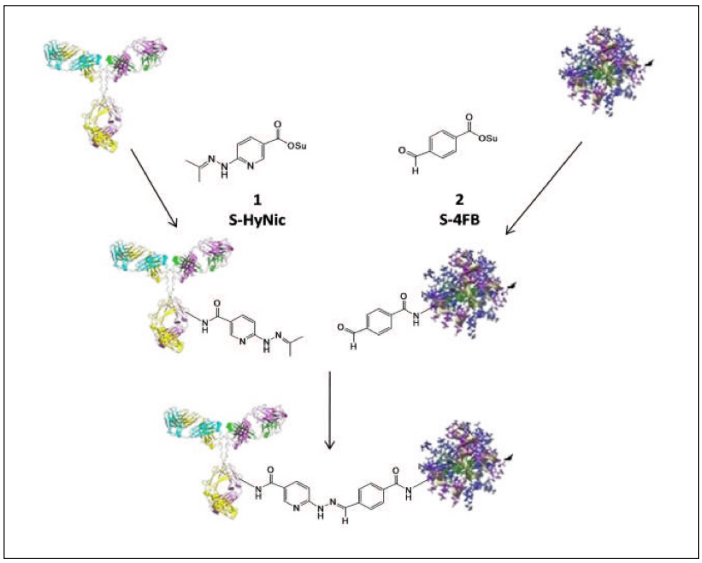


Figure 1: Schematic representation of SoluLINK Bioconjugation chemistry where an antibody is modified with S-HyNic to incorporate HyNic groups and a second protein is modified with S-4FB to incorporate 4FB groups. Conjugate is formed directly by simply mixing the HyNic-modified antibody with the 4FB-modified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

Additional materials required

Reagents

Zeba™ Desalting Columns

Modification Buffer (10X)

Conjugation Buffer (10X)

Anhydrous DMF

2-Sulfobenzaldehyde

Equipment

Variable-speed bench-top microcentrifuge

Spectrophotometer or Plate Reader

1.5 mL microcentrifuge tubes

Modification Procedure

A. Desalting

1. Desalt/buffer exchange the protein into Maleimide Reaction Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 6.5). This solution may be prepared by adjusting 1X Modification Buffer from pH 8.0 to pH 6.5. If needed, refer to the Protein Desalting Protocol.

   Notes:

   a) High-level buffering capacity, i.e. 100 mM phosphate, is necessary for successful modification.

   b) For desalting proteins, Zeba Desalting Columns are recommended.

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Continued from page 1.

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 – 4.0 mg/mL in Maleimide Reaction Buffer, pH 6.5, if necessary.

C. Prepare MHPH/DMF stock solution

1. Prepare a stock solution of MHPH in anhydrous DMF (or DMSO) by dissolving 2 – 4 mg of MHPH in 100 µL anhydrous DM.

D. Modification of a protein

1. In most cases, a reduction step is required to cleave disulfide bonds prior to modification with MHPH. A typical procedure is to add one-tenth volume of 10 mM TCEP in water to the buffer-exchanged protein. Alternative reducing agents such as DTT or 2-mercaptoethanol may also be used, but these must be completely removed by desalting prior to adding MHPH. If the protein or surface being modified already contains free thiols, this step may not be required.
2. Using Table 1 as a guide, aadd the required volume of MHPH/DMF to the protein solution. Refer to the Protein Modification Calculator, if needed.

IgG Concentration (mg/mL)	MHPH Mole Equivalents Added	Ratio of incorporated HyNic/Protein (MSR)
1.0	20 30	5.3 7.8
4.0	15 20 25	4.6 6.1 7.8

Table 1: The number of HyNic groups incorporated on an antibody is dependent on the number of mole equivalents MHPH added, the number of thiol groups available, and the protein concentration.

3. Allow the reaction to incubate at room temperature for 1–2 hours.

E. Desalting procedure

1. 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 modification level

1. The molar substitution ratio (MSR) can be determined using a colorimetric reaction outlined in Figure 2. 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.

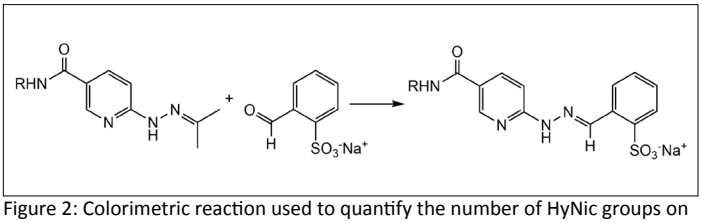

Figure 2: Colorimetric reaction used to quantify the number of HyNic groups on a biomolecule.

2. 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

Troubleshooting Guide