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PROTEIN-PROTEIN CONJUGATION KIT

SKU: S-9010-1

DESCRIPTION

The Protein-Protein Conjugation Kit is designed to easily and efficiently conjugate any two proteins. This kit is flexible so that researchers with little or no bioconjugation experience can make their own custom protein-protein conjugates to suit their research needs. It includes all of the necessary components, including S-HyNic and S-4FB, for the rapid and specific crosslinking of any two proteins.

The molecular weight range for proteins to be conjugated with this kit is 25,000 to 900,000 Daltons. to The molecular weight range for proteins to be conjugated with this kit is 25–950 kDa.

SPECIFICATIONS

Reactivity	Amine
Storage Instructions	2° – 8°C – Do Not Freeze

For research use only. Not intended for therapeutic or diagnostic use in animals or humans.

Applications	In Situ Proximity Ligation
Conjugate	Protein
Label	Protein

KIT COMPONENTS

- S-HyNic (2 x 1.0 mg)
- S-4FB (2 x 1.0 mg)
- 10X Modification Buffer (1.5 mL)
- 10X Conjugation Buffer (1.5 mL)
- 10X TurboLINK™ Catalyst Buffer (1.5 mL)
- 7kDa, 0.5 mL Thermo Scientific™ Zeba™ Column (10)
- Anhydrous DMF (2 x 1.5 mL)
- 2-Hydrazinopyridine (2-HP) reagent (0.5 mL)
- 0.5mM 2-Sulfobenzaldehyde (2-SB) (0.5 mL)
- 2 mL Collection Tube (10)
- 7kDa, 2 mL Zeba™ Column (2)
- 10X PBS (1.5 mL)

TECHNICAL INFORMATION

The ChromaLINK Reaction:

ChromaLINK bioconjugation technology is based on the formation of a stable bis-arylhydrazone formed from an aromatic hydrazine and an aromatic aldehyde. S-HyNic (succinimidyl 6-hydrazinonicotinate acetone hydrazone, SANH) is used to incorporate aromatic hydrazine moieties on biomolecules. S-HyNic is an amine-reactive reagent that directly converts amino groups on biomolecules and surfaces to HyNic groups. S-4FB (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 bis-arylhydrazone bond is stable up to 92℃ and pH 2.0-10.0. Due to lability of the immunoreactivity of antibodies at low pH, i.e. < 5.0, the recommended pH for antibody 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, reducing agents, or metals are required in the preparation of conjugate.

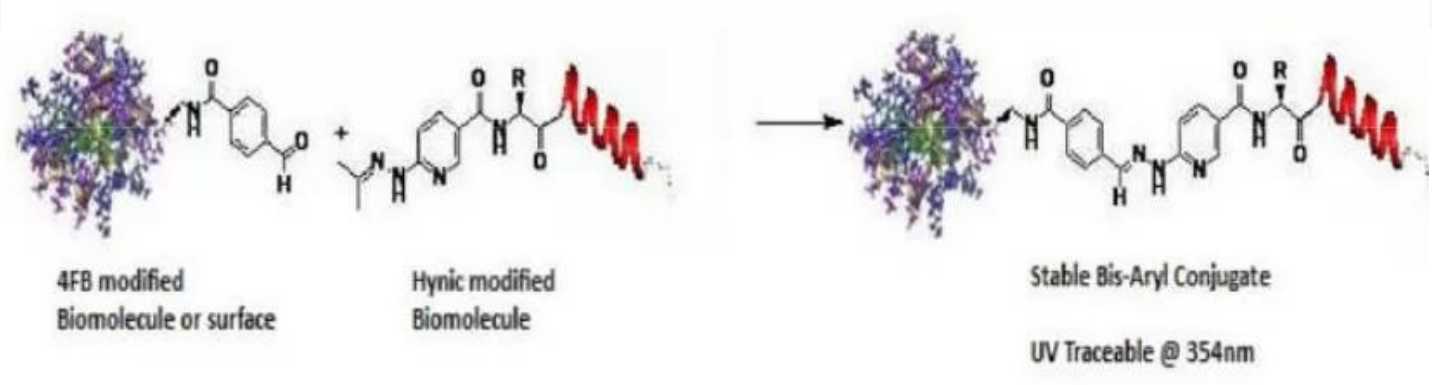

Figure 1: Linking chemistry behind ChromaLINK bioconjugation technology.

Fastest, most efficient:

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 aniline to the reaction mixture converts >95% of the antibody to conjugate in 2 hours using 1-2 mole equivalents of second protein.

Traceable modification:

Reproducibility of any reaction is dependent on accurate characterization of all components. As both HyNic and 4FB are aromatic, their incorporation can be readily quantified using colorimetric assays.

Traceable conjugation:

The HyNic-4FB conjugate bond is chromophoric. It absorbs light at 354 nm and has a molar extinction coefficient of 29,000 L/(mol*cm).

Furthermore, compared to previous methods, the HyNic/4FB technology offers the following practical advantages:

1) **The reaction goes to completion:** In all previous bi-functional linker-based conjugations, the reaction never went to completion, i.e. there was always unconjugated limiting protein in the final product. The HyNic-4FB conjugation couple catalyzed by aniline yields more than 95% conjugate.

2) **The reaction is efficient:** The reaction is very stoichiometrically efficient as input of only 1-2 moles of second protein/mole first protein is required for complete conversion to conjugate.

3) **The conjugate bond is extremely stable:** the bis-arylhydrazone conjugate bond is stable up to 92℃ and pH 2.0-10.0.

4) **The reaction conditions are extremely mild and do not cause antibody denaturation:** 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 metals, oxidizing, or reducing reagents are required.

5) **The conjugation is traceable spectrophotometrically:** the HyNic-4FB conjugate bond is chromophoric; it absorbs light at 354 nm and has a molar extinction coefficient of 29,000 L/(mol*cm).

6) **The modifications of both the HyNic moiety on the protein and the 4FB moiety on the protein is quantifiable using a colorimetric assay:** the reproducibility of any reaction is dependent on accurate characterization of all components. The Molar Substitution Ratio (MSR; i.e. the number of HyNic groups incorporated per protein) can be quantified colorimetrically as a reaction with 2-sulfobenzaldehyde yields a chromophoric product that absorbs at 350 nm with a molar extinction coefficient of 28,500 L/(mol*cm). The MSR of 4FB groups can be determined colorimetrically by its reaction with 2-hydrazinopyridine forming a hydrazone that absorbs at 348 nm with a molar extinction coefficient of 24,500 L/(mol*cm). This kit contains all the reagents necessary to determine both MSRs. Procedures to guide users through this process are given in the protocol below.

The Keys to Successful Conjugation

The following are three crucial requirements that must be fulfilled for a reproducibly successful preparation of a protein-protein conjugate using the SoluLINK bioconjugation technology:

1. **Desalting:** prior to modification, the starting proteins must be thoroughly desalted, removing all amine contaminants and exchanging the proteins into 1X Modification Buffer.

2. **Protein concentration:** the recommended protein concentrations must be adhered to in all steps.

3. **Molar substitution ratio:** the molar ratio of HyNic on the protein and 4FB on the protein must be determined and within the desired range before continuing to the next step.

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DOCUMENTS

- User Guide
- Bioconjugation White Paper
- Safety Data Sheet
- P-9002 – Antibody / R-Phycoerythrin Conjugation Calculator
- Troubleshooting Guide – Bioconjugation
- BCA Protein Assay Protocol
- Bradford Assay Protocol
- Download CoA
- Datasheet

GALLERY IMAGES

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