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R-PE ANTIBODY CONJUGATION KIT

SKU: P-9002-002

DESCRIPTION

The R-PE Antibody Conjugation Kit is a flexible alternative to the R-PE Antibody All-in-One™ Conjugation Kit. Each kit contains sufficient material for two reactions of up to 1.3 mg antibody each. Based on SoluLINK® bioconjugation technology, the reactions are rapid and high-yielding, converting nearly 100% of antibody to PE conjugate. This kit is ideal for applications where purification to remove excess R-PE is either not required, or purification will be carried out by chromatographic methods (such as size exclusion FPLC).

SPECIFICATIONS

Reactivity	Amine
Unit Size	1 Kit
Storage Instructions	2° – 8° C — Do Not Freeze
Conjugate	Antibody

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

Label	R-PE

KIT COMPONENTS

- S-HyNic (2 x 1.0 mg)
- 4FB-R-PE (2 x 3.2 mg)
- 10X Modification Buffer (1.5 mL)
- 10X Conjugation Buffer (1.5 mL)
- 10X TurboLINK™ Catalyst Buffer (1.5 mL)
- Anhydrous DMF (1.5 mL)
- 0.5 mL Thermo Scientific™ Zeba™ Column (4)
- 2 mL Collection Tube (12)
- 2-Sulfobenzaldehyde, 2-SB (10 mg)
- 10X MES Buffer (1.5 mL)
- 2 mL Zeba™ Column (2

TECHNICAL INFORMATION

R-PE-Antibody Conjugation Kit Using the SoluLINK bioconjugation Technology

The SoluLINK technology can be used to more easily and efficiently prepare R-PE-antibody conjugates as compared to alternative (maleimide/thiol-based) protocols. Other conjugation methods expose thiols on antibodies by DTT reduction of disulfide bonds, which cleaves the antibody into a variety of species. The SoluLINK bioconjugation technology, however, gently incorporates HyNic moieties on the intact antibody.

This technology is superior to the maleimide/thiol-based method in the following ways:

- **Efficiency**: Greater than 95% of antibody is converted to conjugate and only 1-1.5 molar equivalents of R-PE is required per mole of antibody to produce the conjugate.
- **Ease of purification:** In most cases, the percent conversion of free antibody to conjugate is >95. Therefore it is only necessary to remove the excess R-PE to obtain a purified conjugate and in many applications, purification is not necessary.
- **Reaction Control:** The level of polymerization, and therefore the brightness of the conjugate, can be controlled by adjusting the level of HyNic modification on the antibody. This is particularly helpful in some applications, such as flow cytometry, where a

heterodimer product is preferred.

Introduction to SoluLINK Bioconjugation Technology

The SoluLINK bioconjugation technology is based on the formation of a stable bond formed by the reaction of an aromatic hydrazine and an aromatic aldehyde (Figure 1). S-HyNic is an amino-reactive modification reagent that directly converts amino groups on biomolecules and surfaces to HyNic groups. S-HyNic 1 (succinimidyl 6-hydrazinonicotinate acetone hydrazone, SANH) is used to incorporate aromatic hydrazine linkers on biomolecules. 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 R-PE leads to the formation of the conjugate via a bis-arylhydrazone bond. The bis-aryl hydrazone bond is stable to 92°C and pH 2.0-10.0. The recommended pH for antibody conjugation is 6.0.

Advantages of the HyNic-4FB conjugation couple include:

- **High Yielding**: Yields of conjugate are routinely 40-60%, based on starting protein.
- **Efficient**: Dirksen *et al*[*] discovered that aniline catalyzes Schiff's base formation. 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 1-1.5 mole equivalents of second protein as strikingly demonstrated in this product.
- **Stability**: The bis-arylhydrazone conjugate bond is stable to 92°C and pH 2.0-10.0.
- **Mild reaction conditions**: These mild conditions 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, oxidation or reducing reagents are required.
- **Spectrophotometric Traceability**: The HyNic-4FB conjugate bond is chromophoric, it absorbs at 354 nm and has a molar extinction coefficient of 29,000 L/(mol*cm). This allows,
    - Real time spectrophotometric monitoring of a conjugate reaction.
    - The ability to visualize the conjugate during chromatographic purification using a UV or photodiode array detector.
    - Quantification of conjugation.

There are three crucial requirements that must be fulfilled for a reproducibly successful preparation of an R-PE Antibody conjugate using the SoluLINK bioconjugation technology:

- **Antibody buffer exchange**: Prior to modification, the starting antibody must be

completely exchanged into Modification Buffer, pH 8.0.

- **Minimum level of HyNic-modification**: The HyNic-antibody molar substitution ratio (MSR) must be >3.5 as determined by a colorimetric assay.
- **HyNic Concentration**: The final concentration of the HyNic-antibody in the conjugation reaction must be >1.5 mg/mL.

References

*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.

LEGAL NOTICE

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

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