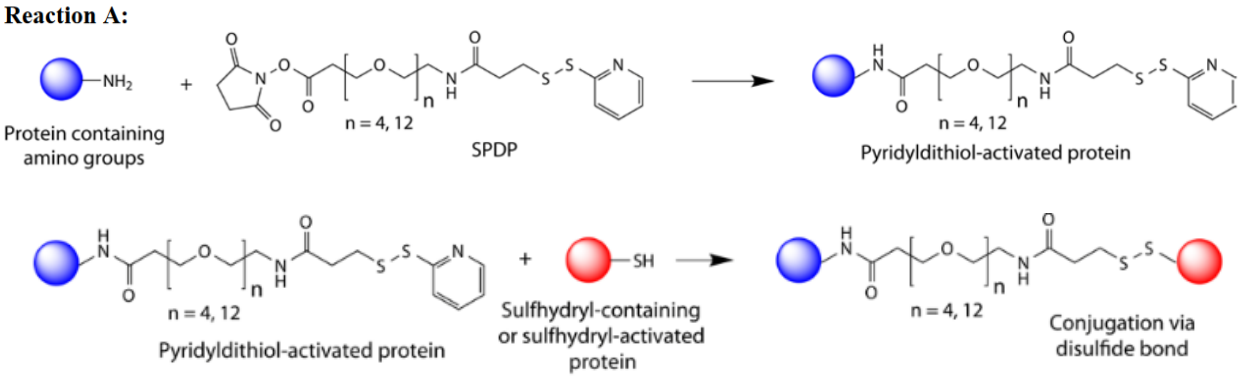
PEG SPDP Reagents



Introduction

SPDP-PEG crosslinkers enable protein conjugations via amine-to-amine or amine-to-sulfhydryl crosslink. The polyethylene glycol (PEG) spacer arms confer great solubility in the reaction media. SPDP-PEG crosslinkers are best dissolved in organic solvent to make the stock solution. before adding to a reaction mixture and SPDP reagents produce disulfide-containing linkages

The amine-reactive portion of SPDP reagents is the N-hydroxysuccinimide (NHS) ester. Conjugation reactions via the NHS ester are most commonly performed at pH 7-8 in phosphate, carbonate/bicarbonate or borate buffers.

The sulfhydryl-reactive portion of SPDP reagents is the 2-pyridyldithio group, which reacts optimally at pH 7-8 in buffers free of thiols. The reaction results in pyridine-2-thione displacement can be determined by measuring the absorbance at 343nm.

The resulting crosslink contains a disulfide and can be cleaved by reduction with dithiothreitol (DTT) or THPP or TCEP. In most cases, crosslinks created using SPDP reagents can be cleaved with 25mM DTT at pH 4.5 without reducing native protein disulfide bonds.

Crosslinking experiments with SPDP reagents are not limited to those involving proteins. Any of a variety of molecules with primary amines and sulfhydryl groups can be modified or crosslinked using an SPDP reagent.

Handling and Storage Information

- Upon receipt store at -20°C protected from moisture.
- Product is shipped at ambient temperature in a sealed bag containing a desiccant. Additionally, the bottle is wrapped with laboratory film to protect the product from moisture and to maintain product integrity.

Procedure of protein A- Protein B conjugation by SPDP-PEG

1. Dissolve 5 mg of SPDP-PEG in 640 µL DMSO or DMF to give a 25 mM stock solution.

2. Dissolve amine-bearing protein A at a concentration of 1-5 mg/mL in 100 mM sodium phosphate buffer, pH 7.2 to pH 8.0, 1 mM EDTA.

3. Add 20 µL of 25 mM SPDP-PEG solution to 1 mL of the above protein A solution.

4. Allow reaction to proceed for 30-60 minutes at room temperature.

5. Remove unreacted SPDP crosslinker from protein containing solution through gel-filtration.

6. Dissolve Thiol-bearing protein B in buffer (100 mM sodium phosphate pH 7.2 to 8.0, 1 mM EDTA).

7. Add 0.2 to 1.0 molar equivalents of protein B solution to desalted activated protein A.

8. Allow this reaction to proceed for 8 to 16 hours at room temperature

9 if needed, the conjugates can be further purified.