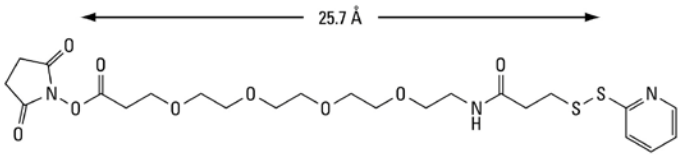
INSTRUCTIONS

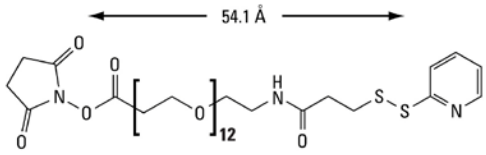
PEG-SPDP Crosslinkers

26128 26129

2340.1

Number	Description
26128	**PEG_4-SPDP**, 100mg Molecular Weight: 559.65 Spacer Arm Length: 25.7Å
26129	**PEG_{12}-SPDP**, 100mg Molecular Weight: 912.07 Spacer Arm Length: 54.1Å





Storage: 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.

Introduction

Thermo Scientific™ PEG-SPDP crosslinkers enable protein conjugations via amine-to-amine or amine-to-sulfhydryl crosslinks. These reagents contain polyethylene glycol (PEG) spacer arms, which confer greater solubility to the crosslinker and the linked proteins compared to crosslinkers having only hydrocarbon spacers; however, the PEG-SPDP crosslinkers have limited aqueous solubility and are best dissolved in organic solvent before adding to a reaction mixture. SPDP reagents produce disulfide-containing linkages that can be cleaved with reducing agents such as dithiothreitol (DTT).

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. Other buffers that do not contain primary amines (or thiols or disulfide reducing reagents – see the sulfhydryl reactivity discussion) are also effective. The rate of reaction and NHS-ester degradation by hydrolysis increases with increasing pH; for example, the half-life of the NHS ester is several hours at pH 7 and less than 10 minutes at pH 9.

The sulfhydryl-reactive portion of SPDP reagents is the 2-pyridyldithio group, which reacts optimally at pH 7-8.[1] The reaction results in pyridine-2-thione displacement, the concentration of which can be determined by measuring the absorbance at 343nm (see the Additional Information Section). Reaction buffers must be free of thiols and disulfide reducing agents until quenching or reduction of the 2-pyridyldithio is desired.

There are two basic strategies for forming cleavable protein crosslinks with SPDP reagents, depending on whether one or neither protein possesses sulfhydryl groups (–SH) in addition to primary amines ($–NH_2$) (Figure 1). Both conjugation methods result in crosslinks that contain a disulfide bond in the spacer arm, which can be cleaved by reduction with dithiothreitol (DTT) or other reducing agent. In most cases, crosslinks created using SPDP reagents can be cleaved with 25mM DTT at pH 4.5 without reducing native protein disulfide bonds; however, when preservation of native disulfide bonds is not a concern, cleavage with DTT can be performed most efficiently at pH 7-9.[1]

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 (see Additional Information section).

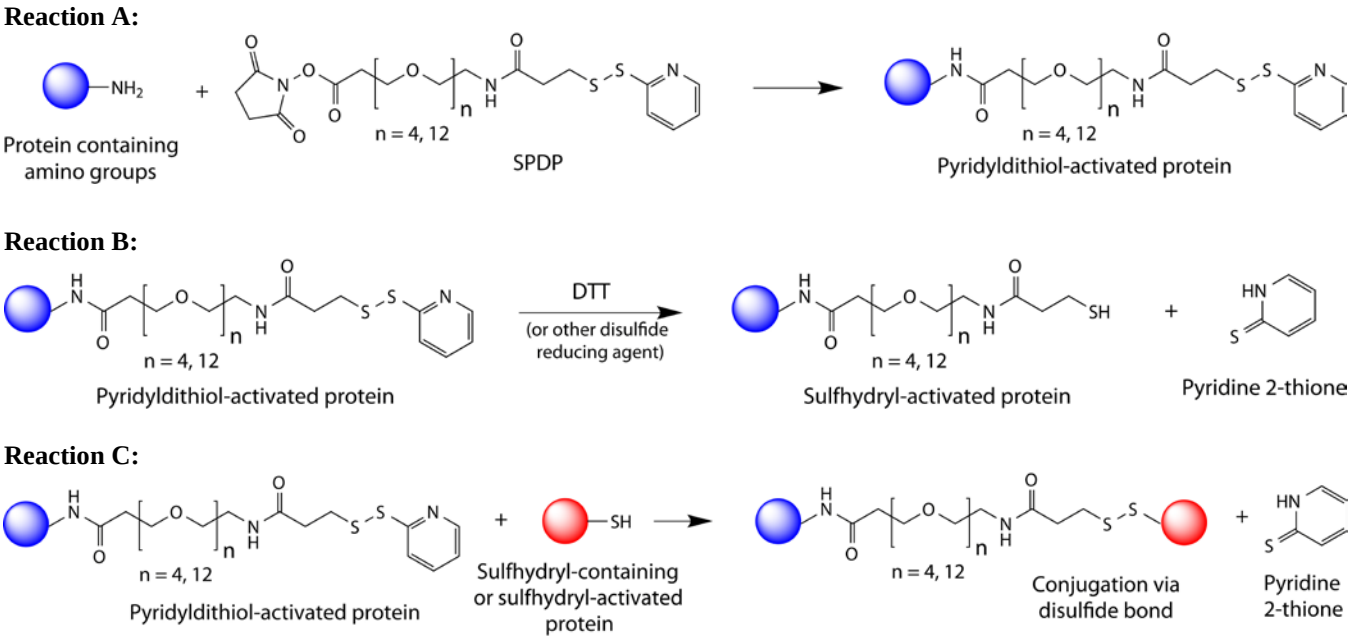


Figure 1. Two protein-to-protein conjugation strategies using an SPDP reagent. If one of the proteins already contains sulfhydryl groups, then SPDP-modify only the protein without sulfhydryls (Reaction A) and proceed to Reaction C. If neither protein contains available sulfhydryl groups, then separately modify both proteins (Reaction A), and then one protein is treated with reducing agent to expose sulfhydryl groups (Reaction B). Finally the two proteins are combined for conjugation (Reaction C).

Procedure for Conjugating a Sulfhydryl-containing Protein

To conjugate primary amines ($-NH_2$) on one protein to sulfhydryls (–SH) present on a second protein, only the first protein must be modified with the SPDP reagent.[2] The SPDP-modified protein is desalted to remove reaction byproducts and excess nonreacted reagent, and the sulfhydryl-containing protein is added to make the final conjugate (Figure 1, Reaction A).

A. Additional Materials Required

- Phosphate-buffered saline with EDTA (PBS-EDTA): 100mM sodium phosphate, 150mM NaCl, 1mM EDTA, 0.02% sodium azide; pH 7.5
- Desalting column (e.g., Thermo Scientific Zeba Spin Desalting Columns, Product No. 89891) sufficient to process 1-2mL samples
- Proteins for conjugation: In the procedure, IgG and β-galactosidase represent the amine-containing and sulfhydryl-containing protein, respectively

B. Procedure

1. Equilibrate the vial of PEG-SPDP to room temperature before opening.
2. Immediately before use, prepare a 20mM solution of PEG-SPDP:
   - For PEG_4-SPDP, dissolve 2mg in 179µL of DMSO or DMF.
   - For PEG_{12}-SPDP, dissolve 2mg in 110µL of DMSO or DMF.
3. Add 25µL of the 20mM PEG-SPDP to 2-5mg of IgG dissolved in 1mL of PBS-EDTA. Incubate for 30 minutes at room temperature.
4. Equilibrate a desalting column with PBS-EDTA, and buffer exchange the SPDP-modified IgG to remove reaction byproducts and excess nonreacted SPDP reagent.

5. Add 1-3mg of β-galactosidase (~1-3 moles β-gal per mole of IgG) to the IgG solution and incubate the reaction mixture overnight at room temperature.
6. The proteins (IgG and β-galactosidase in this example) are now conjugated.

Procedure for Conjugating Proteins without Sulfhydryls

To conjugate via primary amines of both proteins, the proteins must be separately modified with SPDP reagent.[3] Using a reducing agent, the pyridine-2-thione group is removed from only one of the modified proteins (Figure 1, Reaction B). After desalting to remove the reducing agent, the resulting sulfhydryl-modified protein and the SPDP-modified protein are combined to make the final conjugate (Figure 1, Reaction C).

A. Additional Materials Required

- Phosphate-buffered saline with EDTA (PBS-EDTA): 20mM sodium phosphate, 150mM NaCl, 1mM EDTA, 0.02% sodium azide, pH 7.5. **Note:** If acetate buffer will be used in section C, do not exceed 20mM sodium phosphate in this buffer. Otherwise, 20-100mM sodium phosphate can be used.
- Acetate buffer: 100mM sodium acetate buffer, 100mM NaCl, pH 4.5
- Desalting columns (e.g., Zeba™ Spin Desalting Columns, Product No. 89891) sufficient to process 1-2mL samples
- Dithiothreitol (DTT), Product No. 20290
- Two proteins to be conjugated

B. SPDP-modify Both Proteins in Separate Reactions

Note: Perform this section of the procedure separately for both proteins.

1. Equilibrate the vial of PEG-SPDP Reagent to room temperature before opening.
2. Immediately before use, prepare 20mM of PEG-SPDP reagent:
   - For PEG_4-SPDP, dissolve 2mg reagent in 179µL of DMSO or DMF.
   - For PEG_{12}-SPDP, dissolve 2mg reagent in 110µL of DMSO or DMF.
3. Add 25µL of the 20mM PEG-SPDP to 2-5mg of protein dissolved in 1mL of PBS-EDTA. Incubate for 30-60 minutes at room temperature.
4. Equilibrate two desalting columns with PBS-EDTA. Buffer exchange the SPDP-modified proteins to remove reaction byproducts and excess nonreacted SPDP reagent. Alternatively, dialyze the samples using a Thermo Scientific Slide-A-Lyzer Dialysis Cassette and concentrate using the Slide-A-Lyzer® Concentrating Solution.

   Note: Both proteins are now SPDP-modified and one of them must be reduced (Section C) before conjugation.

C. Reduce SPDP Disulfides in One Protein

Note: Choose only one of the two SPDP-modified proteins for this section of the procedure. Use the protein whose function or activity is least dependent on native disulfide bonds.

1. Dissolve 23mg DTT in 1mL acetate buffer or PBS-EDTA (makes 150mM solution). Use acetate buffer to avoid reducing native disulfide bonds in the protein.
2. Add 0.5mL DTT solution per 1mL of SPDP-modified protein (results in 50mM DTT). Incubate for 30 minutes.
3. Equilibrate a desalting column with PBS-EDTA, and desalt the protein (now sulfhydryl-modified) to remove the DTT.

D. Conjugate the Activated Proteins

1. Mix the SPDP-modified and sulfhydryl-modified proteins and incubate for 18 hours at room temperature or 4°C.
2. To isolate conjugate from the two unconjugated proteins, use an appropriate size-exclusion chromatography method.

Additional Information

A. Visit the website for additional information on this product including the following items:

- Tech Tip #1: Attach a protein onto glass, silica or quartz surface using a cleavable crosslinker
- Tech Tip #26: Prepare a reversible sulfhydryl-reactive affinity column

B. Protocol For Pyridine-2-Thione Assay to Determine Level of SPDP-modification

1. Dilute 100µL of SPDP-modified and desalted protein to 1mL with PBS.
2. Measure and record the absorbance at 343nm of the protein sample compared to PBS-EDTA blank (test in triplicate).
3. Add 10µL of 15mg/mL DTT to the 1mL SPDP-modified protein sample, mix.
4. After exactly 15 minutes, measure and record the absorbance at 343nm of the reduced sample.
5. Calculate the change in absorbance: ΔA_{343} = (Ave. A_{343} after DTT) – (Ave. A_{343} before DTT)
6. Calculate molar ratio of SPDP-to-protein using the following equation:

$$\frac{\Delta A}{8080} \times \frac{\text{MW of Protein}}{\text{mg/mL of Protein}} = \text{moles of SPDP per mole of protein}$$

Where the value 8080 reflects the extinction coefficient for pyridine-2-thione at 343nm: $8.08 \times 10^3\ M^{-1}cm^{-1}$.

Related Thermo Scientific Products

20290	**DTT (dithiothreitol),** 5g
21650	**Sulfo-LC-SPDP,** 50mg
21651	**LC-SPDP,** 50mg
21857	**SPDP,** 50mg
89891	**Zeba Spin Desalting Columns, 7K MWCO,** 5 columns

References

1. Carlsson, J., *et al*. (1978). Protein thiolation and reversible protein-protein conjugation. *Biochem J* **173:**723-7.
2. Cumber, A.J., *et al*. (1985). Preparation of antibody-toxin conjugates. *Meth Enzymol* **112:**207-25.
3. Neurath, A.R. and Strick, N. (1981). Enzyme-linked fluorescence immunoassays using β-galactosidase and antibodies covalently bound to polystyrene plates. *J Virol Meth* **3:**155-65.