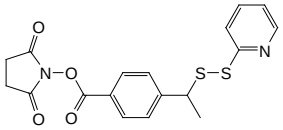
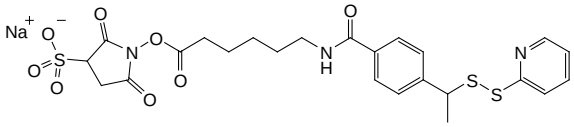
INSTRUCTIONS

SMPT
Sulfo-LC-SMPT

21558 21568

0380.2

Number	Description
21558	**SMPT** (4-succinimidyloxycarbonyl-α-methyl-α-[2-pyridyldithio]toluene), 50mg Molecular Weight: 388.46 Spacer Arm: 11.2Å Formula: $C_{18}H_{16}N_2O_4S_2$  **Storage:** Upon receipt store product desiccated at 4°C. Product is shipped at ambient temperature.
21568	**Sulfo-LC-SMPT** (sulfosuccinimidyl 6-[α-methyl-α-(2-pyridyldithio)toluamido]hexanoate), 50mg Molecular Weight: 603.67 Spacer Arm: 20.0Å Formula: $C_{24}H_{26}N_3NaO_8S_3$  **Storage:** Upon receipt store product desiccated at -20°C. Product is shipped at ambient temperature.

Introduction

Thermo Scientific SMPT is a heterobifunctional sulfhydryl- and amine-reactive cross-linker. SMPT is used often for conjugating a toxin molecule to a monoclonal antibody directed against a cell-surface antigen. These immunotoxins produced with SMPT are highly potent as the cleavable disulfide imparts increased cytotoxicity compared to conjugates without a cleavable bond, such as those produced with SPDP. Also, next to the pyridine-2-thione in the spacer arm are a benzene ring and a methyl group adjacent to a carbon that hinders the disulfide bond, allowing exceptional conjugate stability *in vivo*. SMPT has the added benefit of being more stable to hydrolysis in aqueous solutions than other NHS cross-linkers.

Thermo Scientific Sulfo-LC-SMPT is a water-soluble version of SMPT with an extended spacer arm (20.0Å). The extended spacer arm increases reactivity and minimizes steric hindrance effects, which can occur when conjugating an antibody to a toxin. The NHS ester on Sulfo-LC-SMPT is not as stable in aqueous solutions as it is in SMPT. Additionally, the disulfide bond in Sulfo-LC-SMPT is not as stable as it is in SMPT; however, disulfide bonds formed by Sulfo-LC-SMPT with sulfhydryl-containing molecules are more stable in solution than those formed by SPDP.

Important Product Information

- Dissolve SMPT in an organic solvent, such as acetonitrile, before adding to a buffer. If acetonitrile is incompatible with the application, DMF and DMSO may be used for solubilization provided it is added immediately to the buffer solution containing the protein. SMPT is more stable in acetonitrile than in DMF or DMSO.
- Sulfo-LC-SMPT is water-soluble and can be added as a solid directly to the reaction or prepared as a concentrated solution in the reaction buffer and immediately added to the protein. To prevent condensation in the vial and reagent hydrolysis, allow vial to equilibrate to room temperature before opening.
- Store SMPT stock solutions prepared in acetonitrile frozen and moisture-free. SMPT dissolved in water and stored at room temperature for 16 hours will have a 5% reactivity reduction from NHS ester hydrolysis. Reconstituted Sulfo-LC-SMPT must be used immediately.
- The pyridine-2-thione reacts with free sulfhydryl group(s). Some sulfhydryl-containing molecules oxidize in solution and form disulfide bonds, which cannot react. Thermo Scientific Immobilized Reducing Columns (Product No. 77701) and Immobilized TCEP Disulfide Reducing Gel (Product No. 77712) enable disulfide reduction and protein recovery in the absence of reducing agents. Alternatively, sulfhydryls can be introduced via amine modification using *N*-succinimidyl *S*-acetylthioacetate (SATA, Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101).

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General Crosslinking Procedure

Conjugation efficiency is dependent on protein modification level, buffer composition and pH, protein concentration and reaction time. Modification level depends on the molar ratio of protein to the crosslinker used in the reaction. Increasing the molar ratio also increases incorporation level. Over-modification of a protein can cause precipitation and activity loss. Optimal reaction conditions to use must be determined empirically.

A. Materials Required

- Acetonitrile (Product No. 51101)
- Phosphate Buffered Saline (PBS): 0.1M phosphate, 0.15M sodium chloride; pH 7.2, (Thermo Scientific BupH Phosphate Buffered Saline Packs, Product No. 28372) or other non-amine-containing buffer at pH 6.5-7.5
- PBS-EDTA: PBS containing 10mM EDTA
- Amine-containing protein prepared in PBS
- Sulfhydryl-containing protein prepared in PBS-EDTA
- Desalting columns, such as Thermo Scientific Zeba Spin Desalting Columns, 2mL (Product No. 89889), or dialysis units, such as the Thermo Scientific Slide-A-Lyzer Dialysis Cassette (Product No. 66380; 10K MWCO, 0.5-3mL)

B. NHS-ester Reaction

1. Allow crosslinker vial to completely equilibrate to room temperature before opening. Dissolve SMPT in acetonitrile or Sulfo-LC-SMPT in PBS.
2. Add a volume of crosslinker to the protein to achieve a 2- to 4-fold molar excess of the reagent over the protein. To minimize damage to the protein, do not exceed 10% solvent in the final reaction mixture. For optimal results, use < 5% solvent in the reaction.

   Note: Typical final concentrations of cross-linker to use are 0.1-10mM. It may be difficult to keep the conjugate in solution at crosslinker concentrations above 5mM.
3. Allow the reaction to proceed for 1-2 hours at room temperature or overnight at 4°C. If desired, quench the reaction by adding a solution containing primary amines such as Tris•HCl, pH 8.0.
4. Dialyze sample overnight against PBS-EDTA or use a desalting column equilibrated with PBS-EDTA to remove excess reagent and to exchange the buffer.

C. Pyridine-2-thione Reaction

1. Add the modified protein from Section B to the sulfhydryl-containing protein prepared in PBS-EDTA. React for 12-72 hours at room temperature or 4°C.
2. If desired, quantify the pyridine-2-thione reaction using a spectrophotometer. The pyridine-2-thione leaving group has an absorption maximum at 343nm with an extinction coefficient of $8.08 \pm 0.3 \times 10^3\ M^{-1}\ cm^{-1}$. Quantify conjugation before quenching the reaction with cysteine (Step 3).
3. To inactivate nonreacted pyridine-2-thione groups, add a final concentration of 0.2mM cysteine. This treatment will not affect the disulfide bond in the conjugate.
4. To isolate conjugate, use size exclusion chromatography or ion exchange chromatography.

Additional Information Available on Our Website

- Tech Tip #43: Protein stability and storage
- Tech Tip #3: Determine reactivity of NHS ester biotinylation and crosslinking reagents
- Tech Tip #5: Attach an antibody onto glass, silica or quartz surface

Related Thermo Scientific Products

87717	**Slide-A-Lyzer® G2 Dialysis Cassettes, 2K MWCO, 0.5mL,** 10/pkg
20036	**Bioconjugate Techniques,** 2nd Edition, 785 pages, softcover
23227	**Pierce BCA Protein Assay Reagent Kit**
20291	**DTT (Dithiothreitol), No-Weigh™ Format,** 48/pkg

Product References

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General References

Blakely, D., *et al.* (1987). Effect of chemical deglycosylation of Ricin A chain on the in vivo fate and cytotoxic activity of an immunotoxin composed of ricin A chain and anti-thy 1.1 antibody. *Cancer Res* **47:**947-52.

Cawley, D., *et al.* (1978). Homology between ricin and *Ricinus communis* agglutinin: amino terminal sequence analysis and protein synthesis inhibition studies. *Arch Biochem Biophys* **190(2):**744-55.

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Scott, C., *et al.* (1987). An immunotoxin composed of a monoclonal antitransferrin receptor antibody linked by a disulfide bond to the ribosomal inactivating protein gelonin: potent *in vitro* and *in vivo* effects against human tumors. *JNCI* **79(5):**11163-72.

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Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.