INSTRUCTIONS

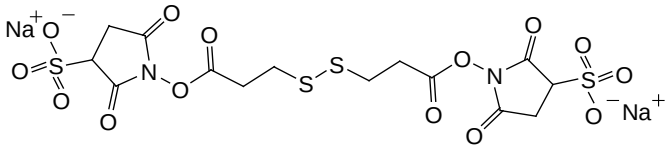
DTSSP

DSP

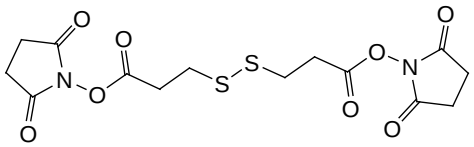
21578 22585 22586

0544.4

Number	Description
21578	**DTSSP** (3,3´-dithiobis[sulfosuccinimidylpropionate]), 50mg Molecular Weight: 608.51 Spacer Arm Length: 12Å Formula: $C_{14}O_{14}S_4H_{14}N_2Na_2$



22585	**DSP** (dithiobis[succinimidylpropionate]), 1g
22586	**DSP,** 50mg Molecular Weight: 404.42 Spacer Arm Length: 12Å Formula: $C_{14}H_{16}O_8N_2S_2$



Storage: Upon receipt store desiccated at 4°C. Product is shipped at ambient temperature.

Introduction

Thermo Scientific DSP is a water-insoluble, homobifunctional *N*-hydroxysuccimide ester (NHS-ester) and DTSSP is its water-soluble analog (Figure 2). These crosslinkers are thiol-cleavable, primary amine-reactive and have been used in many applications (Table 1). NHS-ester reactions with primary amines form covalent amide bonds that results in the release of *N*-hydroxysuccinimide.

DSP is non-sulfonated and, therefore, is non-water-soluble whereas DTSSP is sulfonated and water-soluble. DSP is first dissolved in an organic solvent and added to the aqueous reaction mixture. Because DSP does not possess a charged group, it is lipophilic and membrane-permeable and is useful for intracellular and intramembrane conjugation. DTSSP may be added directly to an aqueous media and is useful for crosslinking cell surface proteins.

Important Product Information

- DSP and DTSSP are moisture-sensitive. Store desiccated at 4-8°C. To avoid moisture condensation onto the product, vial must be equilibrated to room temperature before opening (equilibration may require 30 minutes).
- Reconstitute these crosslinkers immediately before use. The NHS-ester moiety readily hydrolyzes and becomes non-reactive; therefore, do not prepare stock solutions for storage. Discard any unused reconstituted crosslinker.
- Hydrolysis of the NHS ester is a major competing reaction of the acylation reaction. Hydrolysis increases with increasing pH and occurs more readily in dilute protein or peptide solutions.
- Proteins that display biological activity (i.e., enzymes, antibodies etc.) may lose activity upon conjugation, which may be caused by conformational changes of the protein molecule when conjugated. Loss of activity may also occur when the crosslinker modifies lysine groups involved in binding substrate or an antigen.
- To cleave DSP and DTSSP use 20-50mM DTT at 37°C for 30 minutes. For reducing SDS-PAGE sample buffer, use 20-50mM DTT or 2-mercaptoethanol in 2% SDS, 62.5mM Tris base, 10% glycerol at 100°C for 5 minutes.

Procedure for Crosslinking in Solution

Materials Required

- **Crosslinker Solution:** Dissolve DSP in dry DMSO at a 10-25mM. DTSSP may be dissolved in water, buffer or added directly to the sample. Alternatively, prepare DTSSP in 5mM sodium citrate buffer, pH 5.0 and add it drop-wise to the reaction mixture. Discard any unused reconstituted crosslinker.
- **Reaction Buffer:** Phosphate buffered saline (e.g., 0.1M phosphate, 0.15M NaCl; pH 7.2; Product No. 28372); HEPES; bicarbonate/carbonate or borate buffers at pH 7-9 also may be used. Avoid any buffer that contains primary amines (e.g., Tris, glycine, etc.), as they will compete with the cross-linking reaction.
- **Stop Solution:** 1M Tris, pH 7.5 (Tris or glycine can be used to quench the reaction.)

Procedure

1. Prepare the protein sample in Reaction Buffer. If the sample solution contains Tris or glycine, dialyze extensively against the Reaction Buffer.
2. Add crosslinker to the protein sample. Add a 10-fold molar excess of the crosslinker to the protein when the protein concentration is > 5mg/mL. If the protein is < 5mg/mL add a 20- to 50-fold molar excess of the crosslinker. (The crosslinker may be used between 0.25-5mM.)
3. Incubate the reaction mixture at room temperature for 30 minutes or on ice for 2 hours.
4. Add the Stop Solution at a final concentration of 20-50mM and incubate for 15 minutes.

Procedure for Intra- and Extracellular Crosslinking

Note: Use DTSSP for crosslinking molecules at the cell surface as it is membrane-insoluble. Use DSP when crosslinking within the cell.

Materials Required

- **Crosslinker Solution:** Dissolve the DSP in dry DMSO at 10-25mM. DTSSP may be dissolved in water, buffer or added directly to the sample. Alternatively, prepare DTSSP in 5mM sodium citrate buffer, pH 5.0 and add it drop-wise to the reaction mixture. Discard any unused reconstituted crosslinker.
- **Reaction Buffer:** Phosphate Buffered Saline (PBS; e.g., 0.1M phosphate, 0.15M NaCl; pH 7.2; Product No. 28372); HEPES; bicarbonate/carbonate or borate buffers at pH 7-9 also may be used. Avoid any buffer that contains primary amines (e.g., Tris, glycine, etc.), as these buffers will compete with the crosslinking reaction.
- **Stop Solution:** 1M Tris, pH 7.5 (Tris or glycine can be used to quench the reaction).

Procedure

1. Wash cells twice with Reaction Buffer to remove media

   Note: For cell-surface interaction studies, add ligands to the cells and incubate for 1 hour at 4°C.
2. Add the Crosslinker Solution to a final concentration of 1-2mM.
3. Incubate the reaction mixture at room temperature for 30 minutes or on ice for 2 hours.
4. Add the Stop Solution to a final concentration of 10-20mM and incubate for 15 minutes.

Table 1. Applications of Thermo Scientific DSP and DTSSP.

DSP Application	Reference
• Examining spatial relationships of the capside polypeptides of the mengo virion	4
• Studying renal Na^+ and K^+ -ATPase	5
• Nearest neighbor relationships of bovine mitochondrial H^+ -ATP	6
• Producing interactions between protein components of the chemotaxis mechanism in *E. coli*	
• Chemical crosslinking of a-CPI	8
• Identifying crosslinked cytochrome P-450 in rat liver microsomes	9
• Studying the influence of metal ions on prothrombin self-association	10
• Studying glycoprotein topology on intact human red blood cells	11
• Molecular identification of receptors for vasoactive intestinal peptide in rat intestinal epithelium	12
• Characterization of a cell surface receptor for colony-stimulating factor (CSF-2a)	13
• Determining membrane antigens by covalent cross-linking to monoclonal antibodies	14
DTSSP Application	
• Cross-linking the extracytoplasmic domain of the anion exchange channel in intact human erythrocytes	15
• Cross-linking studies on Novikoff ascites hapatoma cytokeratin filaments	16
• Characterization of the B lymphocyte Fc receptor for IgE	17
• Crosslinking platelet glycoprotein lb	18
• Characterization of a membrane ribosome complex in *B. subtilis*	19

Please visit our website for additional information on this product including the following item:

- Tech Tip #3: Determine reactivity of NHS ester biotinylation and crosslinking reagents

Related Thermo Scientific Products

20036	**Bioconjugate Techniques,** 2nd edition, 1202 pages, softcover
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs
20290	**DTT (Dithiothreitol),** 5g
20291	**No-Weigh™ DTT (Dithiothreitol),** 48 × 7.7mg microtubes
35602	**2-Mercaptoethanol,** 10 × 1mL ampules
21580	**BS^3,** 50mg, non-cleavable Sulfo-NHS-ester crosslinker
21555	**DSS,** 1g, non-cleavable NHS-ester crosslinker

Cited References

1. Lomant, A.J. and Fairbanks, G. (1976). Chemical probes of extended biological structures: Synthesis and properties of the cleavable protein cross-linking reagent [^{35}S]dithiobis(succinimidyl propionate). *J Mol Biol* **104:**243-61.
2. Carlsson, J., *et al.* (1978). Protein thiolation and reversible protein-protein conjugation. *N*-succinimidyl 3-(2-pyridyldithio)propionate, a new heterobifunctional reagent. *Biochem J* **173:**723-37.
3. Partis, M.D., *et al.* (1983). Cross-linking of protein by w-maleimido alkanoyl *N*-hydroxysuccinimido esters. *J. Prot. Chem.* **2(3):**263-77.
4. Hordern, J.S., *et al.* (1979). Structure of the mengo virion. *Virology* **97:**131-40.
5. dePont, J.J., *et al.* (1980). Use of mono- and bifunctional group-specific reagents in the study of the renal Na^+-K^+ATPase. *Int J Biochem* **12:**307-13.
6. Joshi, S. and Burrows, R. (1990). ATP synthase complex from bovine heart mitochondria. *J Biol Chem* **265:**14518-25.
7. Chelsky, D. and Dahlquist, F.W. (1980). Chemotaxis in *Escherichia coli:* Association of protein components. *Biochem* **19:**4633-9.
8. Kim, C.G. and Sheffrey, M. (1990). Physical characterization of the affinity purified CCAAT Transcription, a-CPI. *J Biol Chem* **265:**13362-9.
9. Baskin, L.S. and Yang, C.S. (1982). Cross-linking studies of the protein topography of rat liver microsomes. *Biochim Biophys Acta* **684:**263-71.
10. Tarvers, R.C., *et al.* (1982). Influence of metal ions on prothrombin self-association. *J Biol Chem* **257:**10708-14.
11. Schweizer, E., *et al.* (1982). Glycoprotein topology on intact human red blood cells reevaluated by cross-linking following amino group supplementation. *Biochem* **21:**6807-18.

12. Laburthe, M., *et al.* (1984). Molecular identification of receptors for vasoactive intestinal peptide in rat intestinal epithelium by covalent cross-linking. *Eur J Biochem* **139**:181-7.
13. Park, L.S., *et al.* (1986). Characterization of the cell surface receptor for a multi-lineage colony-stimulating factor (CSF-2a). *J Biol Chem* **261:**205-10.
14. Hamada, H. and Tsuro, T. (1987). Determination of membrane antigens by a covalent cross-linking method with monoclonal antibodies. *Anal Biochem* **160:**483-8.
15. Staros, J.V. (1982). *N*-Hydroxysulfosuccinimide active esters: Bis(*N*-hydoxyssuccinimide) esters of two dicarboxylic acids are hydrophilic, membrane impermeant, protein cross-linkers. *Biochemistry* **21:**3950-3955.
16. Staros, J.V. and Kakkad, B.P. (1983). Cross-linking and chymotryptic digestion of the extracytoplasmic domain of the anion exchange channel in intact human erythrocytes. *J Memb Biol* **74:**247-54.
17. Knoller, S., *et al.* (1991). The membrane-associated component of the amphiphile-activated, cytosol-dependent superoxide-forming NADPH oxidase of macrophages is identical to cytochrome b559. *J Biol Chem* **266:**2795-804.
18. Waugh, S.M., *et al.* (1989). Isolation of a proteolitically derived domain of the insulin receptor containing the major site of cross-linking/binding. *Biochem* **28:**3448-55.
19. Jung, S.M. and Moroi, M. (1983). Cross-linking of platelet glycoprotein Ib by *N*-succinimidyl(4-azidophenyldithio)propionate and 3,3'dithiobis(sulfosuccinimidyl propionate). *Biochim Biophys Acta* **761:**152-62.

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