INSTRUCTIONS

Pierce DSP, No-Weigh Format

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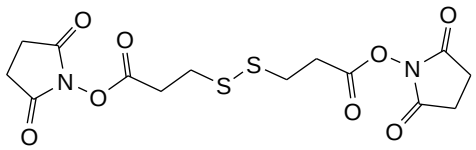
A35393

Number	Description
A35393	**Pierce DSP, No-Weigh Format** (dithiobis[succinimidylpropionate]) 10 × 1mg

Molecular Weight: 404.42
Spacer Arm Length: 12Å
Formula: $C_{14}H_{16}O_8N_2S_2$



For Research Use Only. Not for use in diagnostic procedures.

Storage: Upon receipt store desiccated at 4°C. Product is shipped on ice packs.

Introduction

Thermo Scientific™ DSP is a water-insoluble, homobifunctional *N*-hydroxysuccimide ester (NHS-ester) crosslinker, which is thiol-cleavable, primary amine-reactive and has 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 insoluble in water, so it must first be dissolved in an organic solvent and then 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. A sulfonated analog of DSP (DTTSP) is water soluble.

Thermo Scientific™ No-Weigh™ products are specialty reagents provided in a pre-aliquoted format. The pre-weighed packaging prevents the loss of reagent reactivity and contamination over time by eliminating the repetitive opening and closing of the vial. The format enables use of a fresh vial of reagent each time, eliminating the hassle of weighing small amounts of reagents and reducing concerns over reagent stability.

Important Product Information

- DSP is moisture-sensitive. Store desiccated at 4-8°C. To avoid moisture condensation onto the product, vial must be equilibrated to room temperature before opening.
- Reconstitute 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 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.

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Procedure for Crosslinking in Solution

Materials Required

- **Crosslinker Solution:** Dissolve DSP in dry DMSO. Add 100 µL DMSO to vial to prepare a 25mM solution. Dissolve the solid by repeat pipetting or by replacing the cap and vortexing until a clear solution is obtained. The maximum useable volume of the tube is 800 µL. 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 Intracellular 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 100µL dry DMSO to create a 25mM solution.
- **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
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.

DSP Application	Reference
• Examining spatial relationships of the capside polypeptides of the mengo virion	1
• Studying renal Na^+ and K^+ -ATPase	2
• Nearest neighbor relationships of bovine mitochondrial H^+ -ATP	3
• Producing interactions between protein components of the chemotaxis mechanism in *E. coli*	4
• Chemical cross linking of a-CPI	5
• Identifying crosslinked cytochrome P-450 in rat liver microsomes	6
• Studying the influence of metal ions on prothrombin self-association	7
• Studying glycoprotein topology on intact human red blood cells	8
• Molecular identification of receptors for vasoactive intestinal peptide in rat intestinal epithelium	9
• Characterization of a cell surface receptor for colony-stimulating factor (CSF-2a)	10
• Determining membrane antigens by covalent cross-linking to monoclonal antibodies	11

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

22585	**DSP, 1g**
22586	**DSP, 50mg**
21578	**DTSSP, 50mg, water soluble analog of DSP**
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

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