INSTRUCTIONS

Pierce Premium Grade DSP

PG82081 PG82082

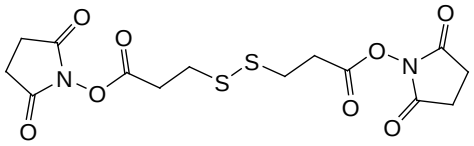
2540.0

Number	Description
PG82081	**Premium Grade DSP** (dithiobis[succinimidylpropionate]), 1g
PG82082	**Premium Grade DSP,** 10g

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™ Pierce™ Premium Grade Reagents are high-quality formulations of selected chemical modification reagents, specially characterized for applications where product integrity and risk minimization are critical. Compared to standard grade equivalents, Pierce Premium Grade Reagents provide more clearly defined quality and product support by including: (a) increased analytical testing and product characterization, (b) greater batch-specific information and quality assurance review, (c) extensive lot sample retention, and (d) change control notification.

Thermo Scientific™ Pierce™ Premium Grade DSP is a water-insoluble, homobifunctional *N*-hydroxysuccimide ester (NHS ester). This crosslinker 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, therefore, is non-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.

Important Product Information

- Pierce Premium Grade DSP is moisture-sensitive. Store desiccated at 4°C. To avoid moisture condensation onto the product, vial must be equilibrated to room temperature before opening (equilibration may require 30 minutes).
- Reconstitute the crosslinker 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 Pierce Premium Grade 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.

Procedure for Crosslinking in Solution

A. Materials Required

- **Crosslinker Solution:** Dissolve Pierce Premium Grade DSP in dry DMSO at 10-25mM 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; Thermo Scientific, Product No. 28372); HEPES; bicarbonate/carbonate or borate buffers at pH 7-9 may also be used. Avoid any buffer that contains primary amines (e.g., Tris, glycine, etc.), as they will compete with the crosslinking reaction.
- **Stop Solution:** 1M Tris, pH 7.5 (Tris or glycine can be used to quench the reaction.)

B. 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 Solution 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 Stop Solution at a final concentration of 20-50mM and incubate for 15 minutes.

Procedure for Intracellular Crosslinking

A. Materials Required

- **Crosslinker Solution:** Dissolve Pierce Premium Grade DSP in dry DMSO at 10-25mM 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; Thermo Scientific, Product No. 28372); HEPES; bicarbonate/carbonate or borate buffers at pH 7-9 may also 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.)

B. Procedure

1. Wash cells twice with Reaction Buffer to remove medium.

   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 Stop Solution to a final concentration of 10-20mM and incubate for 15 minutes.

Table 1. Applications of Thermo Scientific Pierce DSP.

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*	7
• 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

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

PG82083 **Pierce Premium Grade BS^3,** 100mg

PG82084 **Pierce Premium Grade BS^3,** 1g

22585 **Pierce DSP,** 1g

22586 **Pierce DSP,** 50mg

20036 **Bioconjugate Techniques,** 2nd edition, 1202 pages, softcover

28372 **BupH™ Phosphate Buffered Saline Packs,** 40 packs

20290 **Pierce DTT (Dithiothreitol),** 5g

20291 **Pierce DTT (Dithiothreitol), No-Weigh™ Format,** 48 × 7.7mg microtubes

35602 **2-Mercaptoethanol,** 10 × 1mL ampules

21580 **Pierce BS^3,** 50mg, non-cleavable Sulfo-NHS-ester crosslinker

21555 **Pierce DSS,** 1g, non-cleavable NHS-ester crosslinker

References

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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.