INSTRUCTIONS

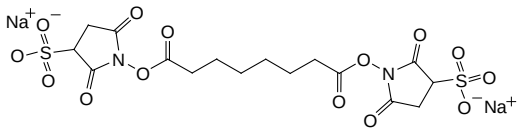
Pierce Premium Grade BS^3

PG82083 PG82084

2541.0

Number	Description
PG82083	**BS^3** (bis[sulfosuccinimidyl] suberate), 100mg
PG82084	**BS^3,** 1g

Molecular Weight: 572.43
Spacer Arm: 11.4Å
Formula: $C_{16}H_{18}N_2Na_2O_{14}S_2$



Storage: Upon receipt store product 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 BS^3 is a water-soluble, homobifunctional *N*-hydroxysuccinimide ester (NHS ester). NHS esters react efficiently with primary amino groups ($-NH_2$) in pH 7 to 9 buffers to form stable amide bonds. The reaction results in the release of *N*-hydroxysuccinimide. Proteins, including antibodies, generally have several primary amines in the side chain of lysine (K) residues and the N-terminus of each polypeptide that are available as targets for NHS-ester reagents.

Pierce Premium Grade BS^3 is supplied as a sodium salt and is water-soluble up to 100mM. BS^3 possesses a charged group and is useful for cell-surface protein crosslinking.

Important Product Information

- Pierce Premium Grade BS^3 is moisture-sensitive. To avoid moisture condensation onto the product, the vial must be equilibrated to room temperature before opening.
- Prepare 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.
- Crosslinking proteins with biological activity (i.e., enzymes, antibodies, etc.) can result in activity loss upon conjugation possibly caused by conformational changes of the molecule. Activity loss also may occur when the crosslinker modifies lysine groups involved in binding substrate or antigen. Adjusting the molar ratios of reagent to the target may overcome activity loss. Alternatively, use a crosslinker that targets a different functional group.
- Hydrolysis of the NHS ester is a competing reaction and increases with increasing pH. Hydrolysis occurs more readily in dilute protein or peptide solutions. In concentrated protein solutions, the acylation reaction is favored.

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Procedure for Crosslinking Proteins

The following protocol is an example application for this product. Specific applications will require optimization. For larger scale coupling, scale accordingly.

A. Materials Required

- Conjugation Buffer: Use a non-amine-containing buffer at pH 7 to 9, such as 100mM sodium phosphate, 0.15M NaCl (Thermo Scientific, Product No. 28372); 20mM HEPES; 100mM carbonate/bicarbonate; or 50mM borate
- Quenching Buffer: 1M Tris•HCl, pH 7.5 (1M glycine or lysine also may be used). Non-reacted reagent can be removed by dialysis or gel filtration.

B. Procedure

1. Prepare protein in Conjugation Buffer.
2. **Note:** Prepare crosslinker immediately before use. Dissolve Pierce Premium Grade BS^3 first in water or 20mM sodium phosphate buffer, as more concentrated buffer salt may interfere with initial solubility of the reagent. Once the BS^3 is dissolved, the solution can be diluted or added to more concentrated buffer solutions without adversely affecting its solubility.

   Weigh 2mg of Pierce Premium Grade BS^3 into a microcentrifuge tube. Add the appropriate volume of buffer based on desired crosslinker concentration. Examples for preparations are as follows:

Buffer Volume to Add to BS^3	Crosslinker Concentration
277µL	12.5mM
140µL	25mM
70µL	50mM
35µL	100mM

3. Add crosslinker to the protein sample. If the protein concentration is greater than 5mg/mL, use a 10-fold molar excess of the crosslinker. For samples < 5mg/mL, use a 20- to 50-fold molar excess of the crosslinker. Use a final concentration of crosslinker at 0.25-5mM.
4. Incubate the reaction mixture at room temperature for 30 minutes or on ice for 2 hours.
5. Quench the reaction using by adding Quenching Buffer to a final concentration of 20-50mM Tris. Alternatively, remove the non-reacted reagent by dialysis or desalting.
6. Incubate the quenching reaction at room temperature for 15 minutes.

Procedure for Extracellular Crosslinking

Crosslinking may be performed on cells in suspension or on adherent cells in culture plates. In the latter situation, diffusion of the crosslinking reagent to all surfaces of the cells will be limited and will occur predominately on the exposed surface. Culture medium must be washed from the cells otherwise amine-containing components will quench the reaction. Using a more concentrated cell suspension is most effective as less reagent will be required in the reaction. Generally, a final concentration of 1-5mM reagent is effective. NHS reactions occur more rapidly with increasing pH; therefore, pH 8.0 is used in the following example so the reaction can be completed quickly.

Note: Use membrane-insoluble BS^3 for crosslinking molecules on the cell surface.

A. Materials Required

- Crosslinker Solution: Immediately before use, dissolve Pierce Premium Grade BS^3 in water or buffer. Pierce Premium Grade BS^3 may be added directly to the cells to decrease the extent of hydrolysis.
- Phosphate-buffered saline (PBS): 20mM sodium phosphate, 0.15M NaCl; pH 8. HEPES, bicarbonate/carbonate or borate buffers between pH 7 and 9 may be used as alternative buffers.
- Quench Solution: 1M Tris, pH 7.5 (Tris or glycine will quench the reaction.)

B. Procedure

1. Suspend cells at ~25 × 10^6 cells/mL in PBS (pH 8.0).
2. Wash cells three times with ice-cold PBS (pH 8.0) to remove amine-containing culture medium and proteins from the cells.

   Note: For cell-surface interaction studies, add ligands to the cells and incubate for 1 hour at 4°C.
3. Add Crosslinker Solution to a final concentration of 1-5mM.
4. Incubate the reaction mixture for 30 minutes at room temperature.

   Note: Performing this incubation at 4°C may reduce active internalization of BS^3.
5. Add Quench Solution to a final concentration of 10-20mM Tris.
6. Incubate the quenching reaction for 15 minutes at room temperature.

Related Thermo Scientific Products

21586	**Pierce BS^3** (bis[sulfosuccinimidyl] suberate), 1g
21580	**Pierce BS^3,** 50mg
21585	**Pierce BS^3, No-Weigh™ Format,** 8 x 2mg microtubes
20036	**Bioconjugate Techniques,** 1202 pages, softcover
66382	**Slide-A-Lyzer™ Dialysis Cassette Kit, 10K MWCO,** 3mL**,** 10-cassette kit
66807	**Slide-A-Lyzer Dialysis Cassette Kit, 10K MWCO,** 12mL, 8-cassette kit
22585	**Pierce DSP** (dithiobis[succinimidylpropionate]), 1g, cleavable NHS-ester crosslinker
PG82081	**Pierce Premium Grade DSP**, 1g
PG82082	**Pierce Premium Grade DSP**, 10g
21578	**Pierce DTSSP** (3,3´-dithiobis[sulfosuccinimidylpropionate]), 50mg, cleavable Sulfo-NHS-ester crosslinker
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 pack

General References

Cox, G.W., *et al.* (1990). Characterization of IL-2 receptor expression and function on murine macrophages. *J Immunol* **145:**1719-26.

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:**2795-2804.

Partis, M.D., *et al.* (1983). Cross-linking of protein by ω-maleimido alkanoyl *N*-hydroxysuccinimido esters. *J Prot Chem* **2(3):**263-77.

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