INSTRUCTIONS

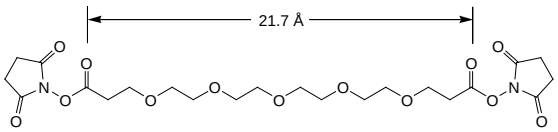
$BS(PEG)_n$

Homobifunctional, amine-reactive crosslinkers with polyethylene glycol (PEG) spacer arms

21581 21582

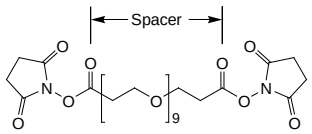
1765.4

Number	Description
21581	**$BS(PEG)_5$,** 100mg Form: Viscous liquid Molecular Weight: 532.50 Spacer Arm: 21.7Å Net Mass Addition: 302.13



$Bis(NHS)PEG_5$
Bis-*N*-succinimidyl-(pentaethylene glycol) ester

21582	**$BS(PEG)_9$,** 100mg Form: Viscous liquid Molecular Weight: 708.71 Spacer Arm: 35.7Å Net Mass Addition: 478.34



$Bis(NHS)PEG_9$
Bis-*N*-succinimidyl-(nonaethylene glycol) ester

Storage: Upon receipt store desiccated at -20°C. Product shipped at ambient temperature.

Introduction

Thermo Scientific™ $BS(PEG)_n$ Reagents are homobifunctional crosslinkers for covalent conjugation between amine-containing molecules. Crosslinkers having polyethylene glycol (PEG) spacers are convenient and useful alternatives to those with purely hydrocarbon spacer arms. PEG spacers improve water-solubility of reagent and conjugate, reduce the tendency of conjugates to aggregate upon storage, and decrease immunogenic response to the spacer itself. By contrast to typical PEG reagents that contain heterogeneous mixtures of different PEG chain lengths, Pierce PEG reagents are homogeneous compounds of defined molecular weight and spacer arm length, providing greater precision in optimization and characterization of crosslinking applications. Homobifunctional crosslinkers containing *N*-hydroxysuccinimide (NHS) esters are often used for low-resolution 3-D studies of protein structure and protein interaction analysis.

Accessible α-amine groups at the N-termini of proteins and peptides and the ε-amine of lysine residues react with NHS esters at pH 7-9 to form covalent amide bonds. The reaction results in the release of NHS. Hydrolysis of the NHS ester is the major competing reaction, the rate of which increases with pH and occurs more readily in dilute protein solutions. NHS ester crosslinking reactions are most commonly performed in phosphate, carbonate/bicarbonate, HEPES and borate buffers. Other buffers may also be used, provided they do not contain primary amines such as Tris or glycine. Using a large excess of Tris or glycine at neutral-to-basic pH can quench the reaction.

Important Product Information

- $BS(PEG)_n$ Reagents are viscous pale liquids that are difficult to weigh and dispense. To facilitate handling, make a stock solution immediately before first use by dissolving the crosslinker in dry (anhydrous, molecular sieve-treated) organic solvent, such as dimethylsulfoxide (DMSO, Product No. 20684). Minimize reagent exposure to moisture because the NHS-ester reactive group is susceptible to hydrolysis. Store unused stock solution in a moisture-free condition (e.g., capped under an inert gas such as argon or nitrogen) at -20°C. Equilibrate reagent vial to room temperature before opening to avoid moisture condensation inside the container. Minimize exposure to air by keeping the stock solution capped by a septum through which reagent can be obtained with a syringe. With proper handling, the stock solution is stable for three months.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

- Avoid buffers containing primary amines (e.g., Tris or glycine) during conjugation because they will compete with the intended reaction. If necessary, dialyze or desalt samples into a buffer such as phosphate-buffered saline (PBS).
- The crosslinker-to-protein molar ratio affects the modification extent of available amine groups and, therefore, crosslinking. This ratio requires optimization to yield the extent of crosslinking best for the specific application.

Procedure for Crosslinking Proteins in Solution

Generally, a 10- to 50-fold molar excess of crosslinker over the amount of amine-containing protein(s) results in sufficient conjugation between proximal amino-groups. Empirical testing of reagent and protein concentrations is necessary to determine optimal conditions for the experiment.

A. Material Preparation

- Conjugation Buffer: Phosphate-buffered saline (PBS, pH 7.2; e.g., Product No. 28372) or other amine-free buffer at pH 7-8 (see Important Product Information).
- Crosslinker Stock Solution: Read the Important Product Information (previous section) before preparing this solution. Prepare a 250mM Crosslinker Stock Solution by dissolving 100mg of $BS(PEG)_n$ (entire contents of vial, ~100µL) in following volumes of dry DMSO. Cap, store and handle the stock solution as directed in the previous section.
    - $BS(PEG)_5$: Add ~650µL DMSO to make 750µL total.
    - $BS(PEG)_9$: Add ~465µL DMSO to make 565µL total.
- (Optional) Quenching Buffer: 1M Tris•HCl, pH 7.5 (1M glycine or lysine also may be used.)
- (Optional) Desalting column (e.g., Thermo Scientific™ Zeba™ Spin Desalting Columns) or dialysis unit (e.g., Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassettes) to separate crosslinked proteins from excess crosslinker and reaction byproducts.

B. Procedure for Soluble Protein Crosslinking

1. Dissolve protein(s) in Conjugation Buffer at 0.1mM (e.g., 5mg in 1mL for a 50kDa protein).
2. Add $BS(PEG)_n$ Reagent to the dissolved protein(s) at 1mM final concentration (= 10-fold molar excess for 0.1mM protein solution) by adding 4µL of Crosslinker Stock Solution per milliliter of protein solution.
3. Incubate the reaction mixture for 30 minutes at room temperature or 2 hours at 4°C.
4. Quench reaction by adding Quenching Buffer at 20-50mM final and incubating for 15 minutes at room temperature. Alternatively, remove the excess non-reacted reagent and reaction byproducts by desalting column or dialysis.

Procedure for Extra-Cellular 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 is limited and occurs predominately on the exposed surface. Culture media must be washed from the cells, otherwise amine-containing components will quench the reaction. Using a concentrated cell suspension is most effective to maximize reagent efficiency. Generally, a final concentration of 1-5mM reagent is effective. The NHS reaction speed increases with increasing pH; therefore, pH 8.0 is used in the following example so the reaction can be completed quickly. $BS(PEG)_n$ Reagents are not membrane-permeable and crosslink molecules only on the cell surface; for intracellular crosslinking experiments, use DSS (Product No. 21655).

A. Material Preparation

- Conjugation Buffer: Phosphate-buffered saline (PBS, 20mM sodium phosphate, 0.15M NaCl, pH 8). HEPES, bicarbonate/carbonate or borate buffer may be used as alternatives.
- Crosslinker Stock Solution: Prepare as directed in Material Preparation Section of the previous procedure. Cap, store and handle the stock solution as directed in the Important Product Information Section.
- (Optional) Quenching Buffer: 1M Tris•HCl, pH 8.0 (1M glycine or lysine also may be used.)

B. Procedure

1. Suspend cells at ~25 × 10^6 cells/mL in PBS (pH 8).
2. Wash cells three times with ice-cold PBS (pH 8) to remove amine-containing culture media and proteins from the cells.
3. Add BS(PEG)$_n$ Reagent to the suspended cells at 1-5mM final by adding 4-20µL of Crosslinker Stock Solution per milliliter of cell suspension.
4. Incubate the reaction mixture for 30 minutes at room temperature. (To decrease active internalization of BS(PEG)$_n$ Reagent, perform this incubation at 4°C or on ice.)
5. Add Quench Solution at 10-20mM final and incubate for 10 minutes.

Related Thermo Scientific Products

21655	**DSS** (disuccinimidyl suberate), 50mg, membrane-permeable NHS-ester crosslinker
21578	**DTSSP** (3,3´-dithiobis[sulfosuccinimidylpropionate]), 50mg, cleavable sulfo-NHS-ester crosslinker
21580	**BS^3** (bis[sulfosuccinimidyl]suberate), 50mg
69576	**Slide-A-Lyzer MINI Dialysis Unit Kit**
66382, 66807	**Slide-A-Lyzer Dialysis Cassette Kits**
89889	**Zeba Spin Desalting Columns, 7K MWCO, 2mL**

General References for NHS-ester Crosslinking

Chang, Z., Kuchar, J. and Hausinger, R.P. (2004). Chemical crosslinking and mass spectrometric identification of sites of interaction for UreD, UreF and urease. *J Biol Chem* **279(15):**15305-13.

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Mattson, G., *et al.* (1993). A practical approach to crosslinking. *Molecular Biology Reports* **17:**167-183.

Partis, M.D., *et al.* (1983). Crosslinking of proteins by omega-maleimido alkanoyl N-hydroxysuccinimide esters. *J Protein Chem* **2:**263-77.

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.

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