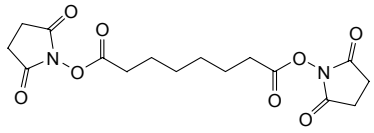
INSTRUCTIONS

DSS and BS^3 Crosslinkers

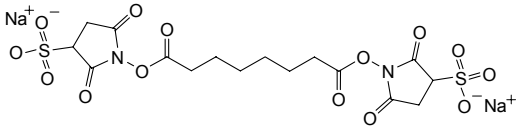
21555 21580 21585 21586 21655 21658 0418.6

Number	Description
21555	**DSS** (disuccinimidyl suberate), 1 g
21655	**DSS,** 50 mg
21658	**DSS, No-Weigh™ Format,** 8 × 2 mg microtubes



Molecular Weight: 368.35
Spacer Arm: 11.4 Å
Formula: $C_{16}H_{20}N_2O_8$

21586	**BS^3** (bis[sulfosuccinimidyl] suberate), 1 g
21580	**BS^3,** 50 mg
21585	**BS^3, No-Weigh Format,** 8 × 2 mg microtubes



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

Storage: Upon receipt store product desiccated at 4°C. Products are shipped at ambient temperature.

Introduction

DSS is a water-insoluble, homobifunctional *N*-hydroxysuccinimide ester (NHS ester), and BS^3 is its water-soluble analog. NHS esters react efficiently with primary amino groups (-NH_2) in pH 7-9 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.

The water-soluble and insoluble forms of NHS-esters have essentially identical reactivity toward primary amines. BS^3 is supplied as a sodium salt and is water-soluble up to 10 mM. DSS is hydrophobic and dissolved in an organic solvent such as DMSO or DMF then added to the aqueous reaction mixture. DSS does not possess a charged group and is lipophilic and membrane-permeable, which makes it useful for intracellular and intramembrane conjugations. Water-soluble BS^3 possess a charged group and is useful for cell-surface protein crosslinking.

Important Product Information

- DSS and BS^3 are moisture-sensitive. To avoid moisture condensation onto the product, vial must be equilibrated to room temperature before opening.
- Prepare 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.
- No-Weigh Microtube Handling: Each of the eight microtubes contains 2 mg of crosslinker. Puncture foil with a pipette tip and add DMSO or DMF to the DSS or aqueous buffer to the BS^3. Store the microtube strip in the foil pouch provided. Used microtubes may be cut from the unused microtubes and discarded.
- 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.

Procedure for Crosslinking Proteins

The following protocol is an example application for this product. Specific applications will require optimization.

A. Materials Required

- Dry dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF) for use with DSS
- Conjugation Buffer: Use a non-amine-containing buffer at pH 7-9, such as 20 mM sodium phosphate, 0.15 M NaCl (Product No. 28372); 20 mM HEPES; 100 mM carbonate/bicarbonate; or 50 mM borate
- Quenching Buffer: 1 M Tris•HCl, pH 7.5 (1 M 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. Prepare crosslinker immediately before use. Dissolve BS^3 first in water or 20 mM 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. Prepare DSS by dissolving in DMSO or DMF. Examples for preparations are as follows:
   - **Microtubes:** Each No-Weigh Microtube contains 2 mg of crosslinker.
   - **Powder:** Weigh 2 mg of crosslinker into a microcentrifuge tube.

Solvent volume to add to 2 mg DSS	Buffer volume to add to 2 mg BS^3	Crosslinker Concentration
432 µl*	277 µl*	12.5 mM
216 µl	140 µl	25 mM
108 µl	70 µl	50 mM
54 µl	35 µl	100 mM

*The maximum volume that can be added to the No-Weigh Microtube is 220 µl.

3. Add crosslinker to the protein sample. If the protein concentration is greater than 5 mg/ml, use a 10-fold molar excess of the crosslinker. For samples < 5 mg/ml, use a 20- to 50-fold molar excess of the crosslinker. Use a final concentration of crosslinker at 0.25-5 mM.
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 20-50 mM Tris. Alternatively, remove the non-reacted reagent by dialysis or desalting.
6. Incubate the quenching reaction at room temperature for 15 minutes.

Procedure for Intra- and 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 media 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 1-5 mM 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. Use DSS when crosslinking within the cell is required.

A. Materials Required

- Crosslinker Solution: Immediately before use, dissolve the DSS in dry DMSO at 10-25 mM. Dissolve BS^3 in water or buffer. BS^3 may be added directly to the cells to decrease the extent of hydrolysis.
- Phosphate-buffered Saline (PBS): 20 mM sodium phosphate, 0.15 M NaCl; pH 8. HEPES, bicarbonate/carbonate or borate buffers between pH 7 and 9 may be used as alternative buffers.
- Quench Solution: 1 M 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 media 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 the DSS or BS^3 solution to a final concentration of 1-5 mM.
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 the Quench Solution to a final concentration of 10-20 mM Tris.
6. Incubate the quenching reaction for 15 minutes at room temperature.

Related Thermo Scientific Products

20036	**Bioconjugate Techniques,** 1202 pages, softcover
66382, 66807	**Slide-A-Lyzer® Dialysis Cassette Kits,** for 0.5-3 ml and 3-12 ml sample volumes, respectively
22585	**DSP** (dithiobis[succinimidylpropionate]), 1 g, cleavable NHS-ester crosslinker
21578	**DTSSP** (3,3´-dithiobis[sulfosuccinimidylpropionate]), 50 mg, cleavable Sulfo-NHS-ester crosslinker
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 pack, each pack yields 500 ml of 0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.2 when reconstituted with 500 ml water

References

Partis, M.D., *et al.* (1983). Cross-linking of protein by ω-maleimido alkanoyl *N*-hydroxysuccinimido esters. *J. Prot. Chem.* **2(3):**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:**2795-2804.

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