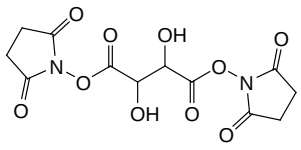
INSTRUCTIONS

DST

20589

0546.3

Number	Description
20589	**DST** (disuccinimidyl tartrate), 50mg Molecular Weight: 344.24 Spacer Arm: 6.4Å Formula: $C_{10}H_{12}N_2O_{10}$



Storage: Upon receipt store DST desiccated at 4°C. Reagent is shipped at ambient temperature.

Introduction

Thermo Scientific DST is a homobifunctional crosslinker that contains amine-reactive *N*-hydroxysuccinimide (NHS) ester groups and is periodate cleavable. DST is commonly used for conjugating radiolabeled ligands to cell surface receptors. DST must first be dissolved in an organic solvent, such as DMSO or DMF, then added to the aqueous reaction mixture. DST is lipophilic, membrane-permeable and does not possess a charged group, which makes it useful for intracellular and intramembrane protein conjugation.

NHS esters react with primary amino groups (-NH_2) present on the side chain of lysine (K) residues and the N-terminus polypeptides. The reaction proceeds efficiently in pH 7-9 buffers to form stable amide bonds and results in the release of *N*-hydroxysuccinimide. Hydrolysis of the NHS ester is a competing reaction and increases with increasing pH. Hydrolysis occurs readily in dilute protein solutions; in concentrated protein solutions, the acylation reaction is favored.

Important Product Information

- DST is moisture-sensitive. Equilibrate vial to room temperature before opening to avoid moisture condensation.
- Prepare DST 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.
- Use a non-amine-containing reaction buffer at pH 7-9 such as 20mM sodium phosphate, 0.15M sodium chloride (Product No. 28372); 20mM HEPES; 100mM carbonate/bicarbonate; or 50mM borate (Product No. 28384). Do not use buffers that contain Tris or glycine, as they will complete with the intended reaction.
- DST contains a central *cis*-diol that can be cleaved with 0.015M sodium *meta*-periodate (Product No. 20504).
- Tris (i.e., 1M Tris, pH 7.5), glycine or lysine can be used to quench NHS-ester reactions. Alternatively, remove non-reacted crosslinker by dialysis or gel filtration.

General Procedure for Crosslinking Proteins

1. Prepare the protein in reaction buffer (See Important Product Information Section). If the protein solution contains Tris or glycine, dialyze extensively against the reaction buffer.
2. Prepare a 10-fold molar excess of crosslinker if the protein is > 5mg/mL or a 20- to 50-fold molar excess if the protein is < 5mg/mL. Use a final crosslinker concentration at 0.25-5mM. Dissolve DST in DMSO at 10-25mM. Use the least amount of solvent as possible (1-10%) in the final reaction to minimize detrimental affects to the protein.
3. Add crosslinker to the protein sample. Incubate reaction at room temperature for 30 minutes or on ice for 2 hours.
4. If desired, quench the reaction using a final concentration of 20-50mM Tris (pH 7.5) and incubate for 15 minutes at room temperature. Alternatively, remove non-reacted crosslinker by dialysis or gel filtration.

General Procedure for Intracellular Crosslinking

Crosslinking may be performed on suspension or adherent cells; however, diffusion of the crosslinker to all surfaces of adherent cells is limited and occurs predominately on the exposed surface.

1. Prepare phosphate-buffered saline (PBS) containing 20mM sodium phosphate, 0.15M sodium chloride; pH 8. Alternatively, use HEPES, bicarbonate/carbonate or a borate buffer between pH 7 and 9.
2. Suspend cells at ~25 × 10^6 cells/mL in PBS.
3. Wash cells three times with ice-cold PBS to remove amine-containing culture media and proteins. For cell-surface interaction studies, add ligands to the cells and incubate for 1 hour at 4°C.
4. Immediately before use dissolve DST in DMSO at 10-25mM. Add the DST solution to a final concentration of 1-5mM.
5. Incubate cells for 30 minutes at room temperature. Performing incubation at 4°C may reduce active internalization of DST.
6. Quench reaction using a final concentration of 10-20mM Tris (pH 7.5) and incubate for 15 minutes at room temperature.

Additional Information Available on Our Website

- Tech Tip #14: Perform labeling and other reactions in Slide-A-Lyzer® Dialysis Cassettes
- Tech Tip #43: Protein stability and storage
- Tech Tip #6: Extinction coefficients guide
- Tech Tip #20: Dialysis: an overview

Related Thermo Scientific Products

20036	**Bioconjugate Techniques,** 2nd edition, 1202 pages, softcover
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs
66382	**Slide-A-Lyzer Dialysis Cassette Kit, 10K MWCO, 3mL,** 10/pkg
66807	**Slide-A-Lyzer Dialysis Cassette Kit, 10K MWCO, 12mL,** 8/pkg

General References

Bragg, P.D. and Hou, C. (1980). A crosslinking study of the Ca^{2+}, Mg^{2+}-activated adeosine triphosphate of *Escherichia coli*. *Eur J Biochem* **106:**495-503.

Carlsson, J., *et al.* (1978). Protein thiolation and reversible protein-protein conjugation. *N*-succinimidyl 3-(2-pyridyldithio)propionate, a new heterobifunctional reagent. *J Biochem* **173:**723-37.

Farries, T.C. and Atkinson, J.P. (1989). Biosynthesis of properdin. *J Immunol* **142:**842-7.

Park, L.S., *et al.* (1986). Characterization of the cell surface receptor for a multi-lineage colony-stimulating factor (CSF-2a). *J Biol Chem* **261:**205-10.

Smith, R.J., *et al.* (1978). Crosslinking of ubiquinone cytochrome C reductase (Complex III) with periodate-cleavable bifunctional reagents. *Biochemistry* **17:**3719-37.