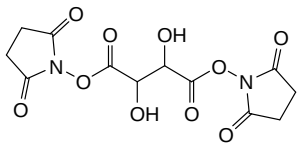
INSTRUCTIONS

DST

20589

0546.2

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



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

Introduction

DST is a homobifunctional cross-linker that contains amine-reactive *N*-hydroxysuccimide (NHS) ester groups and is periodate cleavable. DST is commonly used for conjugating radiolabeled ligands to cell surface receptors. DST must be first 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 cross-linker.
- Use a non-amine-containing reaction buffer at pH 7-9 such as 20 mM sodium phosphate, 0.15 M sodium chloride (Product No. 28372); 20 mM HEPES; 100 mM carbonate/bicarbonate; or 50 mM 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 0.015 M sodium *meta*-periodate (Product No. 20504).
- Tris (i.e., 1 M Tris, pH 7.5), glycine or lysine can be used to quench NHS-ester reactions. Alternatively, remove non-reacted cross-linker by dialysis or gel filtration.

General Procedure for Cross-linking 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 cross-linker if the protein is > 5 mg/ml or a 20- to 50-fold molar excess if the protein is < 5 mg/ml. Use a final cross-linker concentration at 0.25-5 mM. Dissolve DST in DMSO at 10-25 mM. Use the least amount of solvent as possible (1-10%) in the final reaction to minimize detrimental affects to the protein.
3. Add cross-linker 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 20-50 mM Tris (pH 7.5) and incubate for 15 minutes at room temperature. Alternatively, remove non-reacted cross-linker by dialysis or gel filtration.

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General Procedure for Intra-cellular Cross-linking

Cross-linking may be performed on suspension or adherent cells; however, diffusion of the cross-linker to all surfaces of adherent cells is limited and occurs predominately on the exposed surface.

1. Prepare phosphate-buffered saline (PBS) containing 20 mM sodium phosphate, 0.15 M 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 10-25 mM. Add the DST solution to a final concentration of 1-5 mM.
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 10-20 mM Tris (pH 7.5) and incubate for 15 minutes at room temperature.

Additional Information

Please visit the web site for additional information including the following items:

- Tech Tip: Perform labeling and other reactions in Slide-A-Lyzer® Dialysis Cassettes
- Tech Tip: Protein stability and storage
- Tech Tip: Extinction coefficients guide
- Tech Tip: An overview of dialysis

Related Thermo Scientific Products

20002	**Bioconjugate Techniques,** 785 pages, softcover
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs
66382, 66807	**Slide-A-Lyzer® Dialysis Cassette Kits,** for 0.5-3 ml and 3-12 ml sample volumes, respectively

References

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