

DST

20589

0546.2

Number

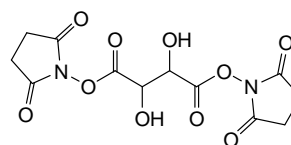
20589

Description

DST (disuccinimidyl tartrate), 50 mg

Molecular Weight: 344.24

Spacer Arm: 6.4 Å

Formula: C₁₀H₁₂N₂O₁₀**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*-hydroxysuccinimide (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 (-NH₂) 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 compete 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.

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 $\sim 25 \times 10^6$ 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

- Bragg, P.D. and Hou, C. (1980). A crosslinking study of the Ca²⁺, Mg²⁺-activated adenosine 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-pyridylthio)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.

Sulfo-NHS Technology is protected by U.S. Patent #s 6,407,263, 5,872,261, 5,892,057 and 5,942,628.

Slide-A-Lyzer® Dialysis Cassette Technology is protected by U.S. Patent # 5,503,741 and other patent pending.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2010 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.