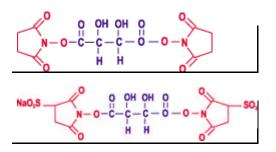


# **DST, Sulfo-DST Homobifunctional oxidizer cross-linkers**

#### **Product Description**

Oxidizer cleavable homobifunctionnal amine reactive crosslinker

| Catalog number:<br>Name:<br>Formula : | UP28068A, 1g<br><b>DST</b><br>DiSuccinimidyl Tartarate<br>$C_{12}H_{12}N_2O_{10}$ , CAS: 70539-4  | 2-3 , <b>M.W.= 344.24</b> |
|---------------------------------------|---|---------------------------|
| Catalog number:<br>Name:<br>Formula : | UP24864A, 100mg UP24864B, 50mg<br><b>Sulfo-DST</b><br>DiSulfoSuccinimidyl Tartarate<br>$C_{12}H_{10}N_2O_{16}S_2Na_2$ , <b>M.W.= 548.32</b> |                           |



Storage :

+4°C (possible at -20°C), protect from moisture and light. (L)

### Introduction

**Cross-linkers** are chemical reagents used to conjugate molecules together by a covallent bound. Several atoms separate the 2 molecules, forming the 'spacer arm'. The conjugate associates the characteristics and biological activities of each component.

Cross-linkers have become important tools for the preparation of conjugates used in a lot of immunotechnologies, and for protein studies (structure, interactions, activity, degradation...). **Homobifunctionnal** cross-linkers present 2 identic reactivities. The choice of the reactivities is determinant to the design of the right conjugate. EGS crosslinker react toward amines, through the succinimide group, and contains a **oxidizer cleavable** linkage.

Uptima offers a high quality DST and it's sulfonated form to answer the needs of coupling proteins and peptides for biological studies and immunoassays like (other cross-linkers are available): (see literature below)

- Obtention of conjugates for structural studies (receptors, ligand interactions...)
- Reticulation of proteins (complexes) in solution or immobilized (receptors)
- Obtention of oligomeric conjugates : poly-peptides
- Immobilization on polystyrene or glass surfaces for immunoassays and biosensors
- Graffting peptides onto gels for chromatography separations
- Graffting haptens onto cells and particules (beads) for diagnostics...

## **Scientific and Technical Information**

- The chemical group N-hydroxysuccinimydyl (NHS) reacts in aqueous phase on primary (–NH2) and secondary amines (=NH) (in fact on its deprotonated form), optimally at neutral pH or higher : amines present in proteins (Lys aminoacid) and in a lower proportion on NH2 located in terminal peptidic chains. The reaction occurs in few minutes in organic media at room temperature, and also in aqueous buffers but in competition with hydrolysis, that increases with pH, and with the high dilutions of the molecule that should be coupled. The reaction with amines occurs typically at pH6.5-8.5 in lhour. n-HydroxySuccinimide is released and should usually be removed before use of the conjugate.
- The sulfonyl moeity (NaSO3) introduces a negatively charged hydrophilic group, that allows the product not to cross biological membranes. It reacts out-side cells, when the crosslinker reacts also in the the membrane and in the cells. An other interest of the sulfonyl group is to permit the solubilisation of the product directly in aqueous buffers, avoiding the use of organic solvants like DMSO or DMF, which are possibly nocive to cells or applications.
- The spacer arm of DST measures 6.3 Angstroms lenght. It is stable, allowing to prepare and use the conjugate. It can however be cleaved further by mild oxidizer agents, like periodate. DST has also used to modify protein before electrophoresis analysis, and conjugates can then be cleaved by soaking the gel in 0.012M sodium periodate, 0.1% SDS, 0.02M sodium phosphate pH7.0 for 2 hours at room temperature. (Smith 1978)



FT-UP28068



• Examples of protocols are given in the literrature. As guidelines, here are some information fro reversible couplings:

<u>Coupling of proteins in solution</u>: the molecule(s) to be coupled is (are) prepared in PBS (20mM phosphate, 150mM NaCl, pH7.5). Other suitable buffers include HEPES, carbonate and borate (but not Tris) provided the pH is keeped between 7 and 9. Crosslinker is added at 5 to 40 molar excess over the protein. Incubation may last 30min to 1H incubation at room temperature (or 1-2H at +4°C if thermolabile proteins). The crosslinking leads to conjugates (dimers...) and to reticulated forms. If different molecules are mixed, homo and hetero conjugates are obtained.

<u>Immobilization of proteins</u>: a soluble protein is conjugated to an aminated (or protein-coated) support (microplate, gel...). Crosslinker is used at 1-5mM with the protein of interest at 1-5mM.

<u>Cell crosslinking</u> the cells at 1-10% suspension, mixed with the protein to be coupled at 2-10nM, are incubated with 0.5-4mM of crosslinker. +4°C is recommended for many cells, and agitation should be mild but continuous.

<u>Immobilization</u> of proteins: the protein is incubated with the crosslinker on the desired protein that is coated on polystyrene or other support. The concentration of protein and crosslinkers should be detremined depending on protein nature and coating density.

If a precipitate is observed, protein and crosslinker concentrations should be decreased, or DMSO added up to 20% final concentration in the reaction mixture.

A stop reaction may be usefull, for example with 20mM Lysine or with a Tris buffer during 15-30min.

A separation t echnic is usually necessary to isolate conjugates (gelfiltation, dialysis, cell washing...)

Other information regarding NHS reactivity are available (<u>NT-NHS</u>: buffers, conditions of use...).

## **Other Information**

For use in vitro only, not for diagnostic.

For any information, please contact Uptima, or your local distributor. 213 av.J.F.kennedy, 03103 Montlucon, fax :+33(0)4 70 03 82 60, hotline Interbiotech : +33(0)4 70 03 76 06

#### **Related products :**

CelluSep<sup>TM</sup> : dialysis membranes

Other cleavable homobifunctionnal amine reactive crosslinkers:

DSP UP18971 (thiol cleavable), DST UP28068 (oxidizer cleavable), EGS UP280067 (mild alkalin cleavable)...

#### Literature: DST

Bragg P.D. and Hou C.; A crosslinking study of the Ca2+ Mg2+-activated adenosine triphosphate of Escherichia coli; Eur. J. Biochem. 1980, 106-495-503

Farries, T.C. and Atkinson, J.P. (1989). Biosynthesis of properdin. J. Immunol. 142, 842-847.

Park, L.S., Friend, D., Gillis, S. and Urdal, D.L. (1986). Characterization of the cell surface receptor for a multi-lineage colony-stimulating factor (CSF-2).

Smith R.J., apaldi R.A., Muchmore D. and Dahlquist F.; crosslinkinf of ubiquinone cytochrome c reductase (complex III) with periodatecleavable bifunctionnal reagents; Biochemistry 1978, 17, 3719-37

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