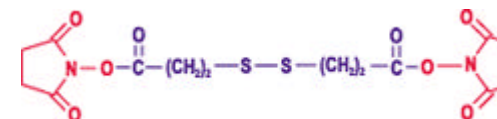


Lomant's reagent : DSP, Sulfo-DSP

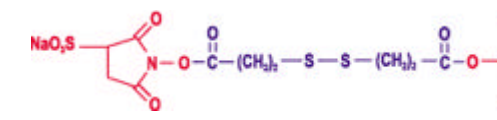
Homobifunctional thiol cleavable cross-linkers

Product description

Catalog number: UP18971A, 1g
Name: Lomant's reagent, DSP, DTSP
Formula : Dithio-bis(Succinimidyl Propionate)
 $C_{14}H_{16}N_2O_8S_2$, CAS: 57757-57-0 , **M.W.= 404.42**



Catalog number: UP434320, 100mg UP43432B, 50mg
Name: Sulfo-DSP
Formula : 3,3'-Dithio-bis(Sulfosuccinimidyl Propionate)
 $C_{14}H_{14}N_2O_{14}S_4Na_2$, **M.W.= 608.5**



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

General Considerations

Cross-linkers are chemical reagents used to conjugate molecules together by a covalent bond. 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...). **Homobifunctional** cross-linkers present 2 identical reactivities. The choice of the reactivities is determinant to the design of the right conjugate. Considering the final result, an important other thing is the nature and length of the spacer. DSP crosslinkers react toward amines, through the succinimide group, and contain a **cleavable** disulfide linkage.

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

- Obtention of conjugates for structural or biological activity studies (receptors, enzymes...)
- Obtention of oligomeric conjugates : poly-peptides
- Immobilization on polystyrene or glass surfaces for immunoassays and biosensors (Darder 1999)
- Grafting peptides onto gels for chromatography separations
- Grafting haptens onto cells for receptor-ligand interaction studies...

Scientific and Technical Information

- The chemical group N-hydroxysuccinimide (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 1hour.

DSP is soluble in DMF, acetone, and chloroform (up to 50mg/ml).

- The sulfonyl moiety (NaSO₃) of Sulfo-DSP introduces a hydrophilic group, that allows the product not to cross biological membranes. An other interest of the sulfonyl group is to permit the solubilisation of the product directly in aqueous buffers, avoiding the use of organic solvents like DMSO or DMF, which are possibly nocive to cells or applications.
- The spacer arm of DSP measures 12.0 Angstroms length. It contains a disulfide bridge is relatively stable, allowing some analysis, but undergoes a slow hydrolysis in aqueous buffers: half-life of ester linkage is ca 5 hours at pH7.0.

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FT-UP18971

- The -S-S- bridge can be cleaved by reducing agents, for example at +37°C with 30mM DTT at pH8.5 within 30minutes. This is taken to good account for the preparation of samples before SDS-PAGE (1-5% SDS, 0.2mM Tris, 10% Glycerol, 5% mercaptoethanol, 100°C, 3min).
- Examples of protocols are given in the literature. As guidelines, here are some information for reversible couplings:

Coupling of proteins in solution: 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 kept 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.

Immobilization of proteins: 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.

Cell crosslinking: 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.

Immobilization 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 determined 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 useful, for example with 20mM Lysine or with a Tris buffer during 15-30min.

A separation technique is usually necessary to isolate conjugates (gel filtration, dialysis, cell washing...)

Other information regarding NHS reactivity are available ([NT-NHS](#); buffers, conditions of use...).

Other Information

For use *in vitro* only, not for diagnostic.

For any information, please contact Uptima, or your local distributor.

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Related products :

MaxiBind™ : activated proteins for immunization and screening conjugates

CelluSep : dialysis membranes

Other cleavable homobifunctional amine reactive crosslinkers:

DSP [UP18971](#) (thiol cleavable), DST [UP28068](#) (oxidizer cleavable), EGS [UP28067](#) (mild alkaline cleavable)...

Literature:

Joshi S. and Burrows R.; AT synthetase complex from bovine heart mitochondria; J. Biol. Chem., 1990, 265, 14518-15252

Lomant, A.J., and Fairbanks G., Chemical probes of extended biological structures: synthesis and properties of the cleavable protein cross-linking reagent [35S]dithiobis(succinimidyl propionate); J. Mol. Biol., 1976, 104, 243-261

Darder M. et al., Dithiobissuccinimidyl propionate as an anchor for assembling peroxidases at electrode surfaces and its application in a H₂O₂ biosensor; Anal. Chem. 1999, 71, 5530-5537

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