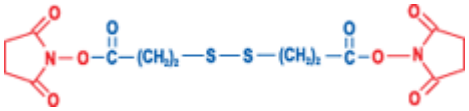
FT-18971A

Lomant's reagent : DSP, Sulfo-DSP
Homobifunctional thiol cleavable crosslinkers

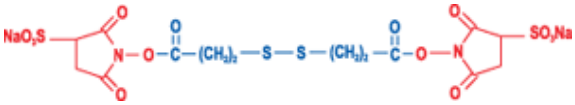
Products Description

Catalog number: UP18971A, 1g
Name: **Lomant's reagent, DSP, DTSP**
Formula : Dithio-bis(Succinimidyl Propionate)



CAS: 57757-57-0 , **M.W.= 404.42**

Catalog number: UP434320, 100mg UP43432B, 50mg
Name: **Sulfo-DSP**
Formula : 3,3'-Dithio-bis(Sulfosuccinimidyl Propionate); DTBSSP; 1-[3-[3-(2,5-dioxo-3-sulfopyrrolidin-1-yl)oxy-3-oxopropyl]disulfanylpropanoyloxy]-2,5-dioxopyrrolidine-3-sulfonic acid



CAS: 81069-02-5; **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 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…). **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** dissulfide 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)

- Preparation of conjugates for structural or biological activity studies (receptors, enzymes…) (Coskun 2004)
- Preparation 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-hydroxysuccinimydyl (NHS)** reacts in aqueous phase on primary ($-NH_2$) 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 NH_2 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.
- The **sulfonyl moeity** (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 solubilization of the product directly in aqueous buffers, avoiding the use of organic solvants like DMSO or DMF, which are possibly deleterious to cells or applications.
  DSP is **soluble** in DMF, acetone, and chloroform (up to 50mg/ml).

- The **spacer arm** of DSP measures 12.0 Angstroms length. It contains a dissulfide bridge that is relatively stable, but undergoes a slow hydrolysis in aqueous buffers: half-life of ester linkage is ca 5 hours at pH7.0.
  The –S-S- bridge can by **cleaved** by reducing agents, for example at +37°C with 30-50mM DTT at pH8.5 within 30minutes (Lomant1976, Laburthe 1984) , or TCEP at pH5.0 within 5min (Han 1994). 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 fro 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.
Crosslinking to monoclonal abs: Hamada, H., Tsuro, T. (1987) Anal. Biochem. 160, 483-488.

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.
Characterizing CSF-2a receptors: Park, L.S., Friend, D., Gillis, S., Urdal, D.L. (1986) J. Biol. Chem. 261, 205-210
Studing glycoproteins in human RBC: Schweizer, E., Angst, W., Lutz, H.V., (1982) Biochem. 21, 6807-6818

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 remove by-products from conjugates (gelfiltation, dialysis, cell washing…)

Other information regarding NHS reactivity is 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 :

-**Desalting** tools: CelluSep dialysis membranes, desalting columns
-**Reducers**: DTT UP284250, TCEP UP242214
-**Activated reactive proteins** for immunization and screening conjugates: MaxiBind™
-Other **crosslinkers** - cleavable homobifunctionnal amine reactive :
DSP UP18971 (thiol cleavable), DST UP28068 (oxidizer cleavable), EGS UP280067 (mild alkaline cleavable)…
- non-cleavable: MAL-PEOx-NHS AL6580; SMCC Plus JQ3870; SANH BL9270,...

Literature:

Darder M. et la., Dithiobissuccinimidyl propionate as an anchor for assembling peroxidases at electrodes surfaces and it's application in a H2O2 biosensor; Anal. Chem. 1999, 71, 5530-5537
Han, J.C., Han, G.Y. (1994) Anal. Biochem. 220, 5-10.
Laburthe, M., Breant, B., Rouyer-Fessard, C. (1984) Eur. J. Biochem. 139, 181-187.
Lomant, AJ., 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
Joshi S. and Burrows R.; AT synthetase complex from bovine heart mitochondria; J. Biol. Chem., 1990, 265, 14518-15252
Coskun Unal, Radermacher, Volker M., Ruiz T., and Gruber G.; Three-dimensional organization of the archeal A1 ATPase from Methanosarcina mazei Go1; JBC 2004-03-30 *[DSP used to investigate the topology and subunit-subunit interactions of the A1 ATPas as a function of nucleotide binding]*

rev. K01E-B12E