FT-79042A

SPDP, lc-SPDP, Sulfo-lc-SPDP Heterobifunctional cross-linkers

Products Information

Heterobifunctional cross-linker for coupling an NH_2-containing molecule with a cleavable spacer.

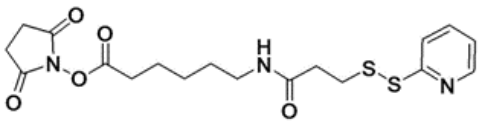
Catalog number: UP79042A, 100mg UP79042B, 50mg
Name: **SPDP**

Formula : N-Succinimidyl-3-(2-PyridylDithio)-Propionate
CAS: 68181-17-9, **M.W.**= 312.37

Spacer: 6.8A

Catalog number: UP88622A , 100mg UP88622B , 50mg
Name: **lc-SPDP**

Formula : N-Succinimidyl-6-(3'-(2-PyridylDithio)-Propionamido)-hexanoate
M.W.= 425.53



Catalog number: UP88621B , 50mg
Sulfo-lc-SPDP

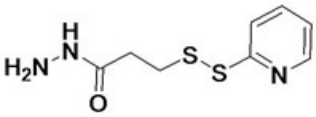
Formula : Sulfosuccinimidyl-6-(3'-(2-PyridylDithio)-Propionamido)-hexanoate
M.W.= 527.57

Spacer: 6.8A

Catalog number: UP97163A , 50mg
Name: **PDPH** (PDP-Hydrazide, SPDP-hydrazide)

Formula : 3-[2-Pyridyldithio]propionyl hydrazide
M.W.= 229.32

Spacer: 9.2A



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

Caution Toxic !

Introduction

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 components.
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…). To that point, heterobifunctional cross-linkers are probably the most interesting, because they present 2 reactivities that allow the conjugation of molecules in a defined manner, avoiding notably the formation of dimeres and polymeres. The choice of 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.
SPDP contains a reactivity toward sulfhydryls, through the pyridylthiol group, and a reactivity toward amines through the succinimide group, (or toward glycoside for **PDPH)**.

Uptima offers a high quality SPDP reagents to answer the needs of coupling proteins and peptides for bio-assays and biotech applications (other cross-linkers are available): to prepare for example,

- carrier-hapten conjugates for immunizations
- labeled affine probes, like antibodies coupled to enzyme, fluorophore-peptides, enzyme-drugs for using as detectors or tracers in immunoassays (ELISA? WB…)
- immobilization: peptides-coated plates or resins, grafting haptens onto cells…
- biologically active conjugates: specific antibody coupled to drugs for immunotargetting techniques, immunotoxins, reticularion of oligomeres or units of complexes proteins for structural studies…

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 competes with hydrolysis, that increases with pH, and with the high dilutions of the molecule that should be derivatized. The reaction is completed usually within 1-2hours (check absorbance at 260nm do not incrise anymore), and should be performed first.
- The chemical group **hydrazide** selectively reacts with carbohydrates oxidized by sodium meta-periodate (or carboxyls when used with EDC). Thus, PDPH protein crosslinker is an excellent choice for crosslinking glycoproteins, glycans, and other polysaccharides.
- The **pyridyl thiol** group reacts specifically at pH7-9 by exchange with sulfhydryl, leaving a pyridin-2-thione group that can be followed up: maximum absorbtion occurs at 343nm with an extinction coefficient of 8.03 . 10^3 M^{-1} cm^{-1} (Struchbury 1975). The formed link includes a –S-S- bound (disulfide).
- The **sulfonyl moeity** ($NaSO_3$) introduces a hydrophilic group, that allows the product not to cross biological membrans. This is particularly useful to modify, in situ on cells, proteins presented outside membrans, and if one wants to avoid the modification of intracellular proteins that may affect further analysis. An other interest of the sulfonyl group is to permit the solubilization of the product directly in aqueous buffers, up to 10mM, avoiding the use of organic solvants like DMSO or DMF, that are possibly nocive to cells or applications.
- The **spacer arm** measures 6.8 (SDPP), or 15.7 (lc-SPDP) Angstroms length. It increases the availability for ligands binding (streptavidin, avidin).
- Proteins containing disulfide bonds can be reduced by DTT (#UP28425) or TCEP (#UP242214) at pH 7-9 for 30min to generate SH before coupling to pyridylthiol group. Alternatively, SH groups can be introduced thanks 2-Iminothiolane (#UP42425) or SATA (#UP84235). Excess of reducer should naturally be removed before reaction with SPDP reagents.
- An other way to conjugate proteins is to derivatize both proteins to be conjugated (SPDP-protein), to reduce one SDPD-protein, desalt it, and allow it then to react with the other for >6 hours at pH7-8.

  protein1 + SPDP → SPDP-protein1 + DTT → SH-SPDP-protein1

  protein2 + SPDP → SPDP-protein2 + SH-SPDP-protein1 → protein2-S-S-protein1

Directions for Use

- ***SPDP and lc-SPDP*** *must be dissolved in DMSO. Uptima recommends not to store the stock solution, because the product is readily subject to hydrolysis. A limited storage may be obtained when using high quality anhydrous DMSO under argon or nitrogen gas at –20°C.*
- ***Sulfo-lc-SPDP*** *can be dissolved directly in distilled water (this solution should be used immediately), or added directly to the proteic solution.*
- ***PDPH*** *is soluble at 14.2 mg/mL in pH 5.5 0.1 M Sodium Acetate.*

Protocol for conjugation of protein with free amine groups using SPDP

This is an example protocol of R-Phycoerythrin to immunoglobulin

1- Prepare the antibody at 5 mg/ml in PBS (NaCl 150 mM, Phosphate 20 mM, pH 7.5):
   - This can be done by dissolving the lyophilized antibody, or by dilution.
   - Check if it contains no other proteins or Tris or other interfering agents. If not, purify, dialyse, or gelfiltrate in the right buffer. Other concentrations can be realised, but the coupling ratio should be slightly increased if the antibody is more diluted.

2- Prepare the crosslinker (SPDP, lc-SPDP) solution at 20mM in DMSO or sulfo-lc-SPDP solution at 20 mM in water. The right quantity can be directly added to the protein solution.

3- Add 15 µL of the solution of NHS-biotin to the antibody (1 ml).

4- Incubate for 1H at room temperature.

5- Dialyses the antibody against PBS+NaN_3 0.01% (Use CelluSep membranes). The biotinylated antibody can be diluted to 1 mg/ml with 0.1% NaN_3 and 20% of glycerol for storage at–20°C or +4°C.

References

References - SPDP

- **Carlsson, J**. *et al*. (1978). Protein thiolation and reversible protein-protein conjugation. N-succinimidyl-3-(2-pyridyldithio)propionate, a new heterobifunctional reagent. *Biochem. J.* 173, 723-737.
- **Cumber A.J**. *et al,* Preparation of antiboy-toxin conjugates. *Meth. Enzymol*. 1985, 112, 207-225
- **Jarvis G**. *et al*., Identification of a major GpVI-binding locus in human type III collagen, *Blood*, 111: 4986 - 4996 (2008) Article
- **Neurath A.R. and Strick N**., Enzyme-linked fluorescence immunoasssays using beta-galactosidase and antibodies covalently bound to polystyrene plates. *J. Virol. Meth*. 1981, 40 219-220
- **Turcatti G**. *et al*., A new class of cleavable fluorescent nucleotides: synthesis and optimization as reversible terminators for DNA sequencing by synthesis, *Nucleic Acids Res*., 36: e25 (2008) Article
- Wang 1997, Rajur 1997, Gordon 1987

References - PDPH

- **Friden**, P.M., et al. (1993). Science 259, 373-377.
- **Greenfield**, R.S., et al. (1990). Evaluation in vitro of adriamycin immunoconjugates synthesized using an acid sensitive hydrazone linker. Cancer Res. 50, 6600-6607.
- **Zara**, J.J., et al. (1991). Anal. Biochem. 194, 156-162.

Other information

Related / associated products

See BioSciences Innovations catalogue and e-search tool.

-Reducers: DTT #UP28425 or TCEP #UP242214

-Useful modifiers: ▪ SATA #84235A, Iminothiolane #42425A ▪ SMCC-hydrazide #BI1281

-Other crosslinkers (non cleavable) and ther conjugation technologies
- ▪ Homobifunctional crosslinkers: NHS-NHS reagents, i.e. NHS-PEO-NHS and SMCC #54940A
- ▪ Homobifunctional crosslinkers: MAL-MAL reagents, i.e. MAL-PEO-MAL #L7736A
- ▪ PhotoActivable (PA) crosslinkers: SH and PA reactive i.e. SCBP #BI1361,...
- ▪ Hydrazone chemistry: Conjugation kit #BL1501 and crosslinkers (SANH #BL9270, MHPH #BL9401)

-Desalting tools: CelluSep dialysis tubings, Desalting gelfiltration columns #UP84874

-Activated proteins for immunization and screening conjugates: KLH, BSA, OVA, MaxiBind™, Activated KLH 86734A

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

For any information, please contat Uptima, or your local distributor.
213 av.J.F.Kennedy, 03103 Montluçon, fax :04 70 03 82 60, hotline Interbiotech : +33 (0)4 70 03 73 06

rev. J04E-B07E