

# SPDP, Ic-SPDP, Sulfo-Ic-SPDP

## Heterobifunctional cross-linkers

### Product Description

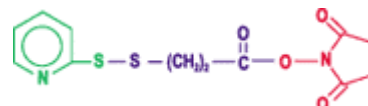
Heterobifunctional cross-linker for coupling an NH<sub>2</sub>-containing molecule and a SH-containing molecule

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

**Name:** **SPDP**

**Formula:** N-Succinimidyl-3-(2-PyridylDithio)-Propionate

CAS: 68181-17-9, C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>S<sub>2</sub>, M.W.= 312.37

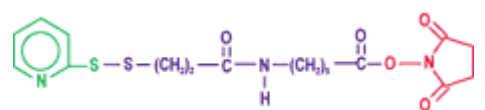


**Catalog number:** UP88622A, 100mg                      UP88622B, 50mg

**Name:** **Ic-SPDP**

**Formula:** N-Succinimidyl-6-(3'-(2-PyridylDithio)-Propionamido)-hexanoate

C<sub>18</sub>H<sub>23</sub>N<sub>3</sub>S<sub>2</sub>O<sub>5</sub>, M.W.= 425.53

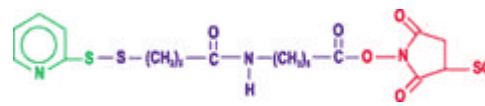


**Catalog number:** UP88621A, 100mg                      UP88621B, 50mg

**Name:** **Sulfo-Ic-SPDP**

**Formula:** Sulfosuccinimidyl-6-(3'-(2-PyridylDithio)-Propionamido)-hexanoate

C<sub>18</sub>H<sub>22</sub>N<sub>3</sub>S<sub>3</sub>O<sub>8</sub>Na, M.W.= 527.57



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

### Introduction

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...). 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 dimers and polymers. 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 one reactivity toward amines, through the succinimide group, and a reactivity toward sulfhydryls, through the pyridylthiol group.

**Uptima** offers a high quality **SPDP** reagents to answer the needs of coupling proteins and peptides for biological and immunoassays like (other cross-linkers are available):

- Obtention of immunogens carrier-hapten
- Obtention of labeled affine probes: for example, antibodies coupled to enzyme for immunoblotting, fluorophore-peptides conjugates for the study of receptors, enzyme-drugs for using as tracers in ELISA...
- Obtention of oligomeric conjugates : conjugates of oriented peptides for immunization, dimeric proteins for structural studies, grafting haptens onto cells...
- Obtention of biologically active conjugates: specific antibody coupled to drugs for immunotargeting techniques, immunotoxins, ...

## 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 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 increase anymore), and should be performed first.
- 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 absorption occurs at 343nm with an extinction coefficient of  $8.03 \cdot 10^3 \text{ M}^{-1} \text{ cm}^{-1}$  (Struchbury 1975). The formed link includes a  $-S-S-$  bound (disulfide).
- The sulfonyl moiety ( $NaSO_3$ ) introduces a hydrophilic group, that allows the product not to cross biological membranes. This is particularly useful to modify, in situ on cells, proteins presented outside membranes, 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 solvents 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) at pH 7-9 for 30min to generate SH before coupling to pyridylthiol group, or SH can be introduced thanks 2-Iminothiolan (#UP42425) or SATA (#UP84235). Excess of DTT 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 SPDP-protein, desalt it, and allow it then to react with the other for >6 hours at pH7-8.
 
$$\begin{array}{l} \text{protein1} + \text{SPDP} \rightarrow \text{SPDP-protein1} \\ \text{protein2} + \text{SPDP} \rightarrow \text{SPDP-protein2} \end{array} \quad + \text{DTT} \rightarrow \begin{array}{l} \text{SH-SPDP-protein1} \\ + \text{SH-SPDP-protein2} \end{array} \rightarrow \text{protein2-S-S-protein1}$$

## Use

Uptima gives some protocol but each application would be optimised according to proteins.

- **SPDP and lcSPD** 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 gaz at  $-20^\circ\text{C}$ . **Sulfo-lc-SPDP** can be dissolved directly in distilled water (this solution should be used immediately), or added directly to the proteic solution.

### Protocol for conjugation of protein with free amine groups

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  $\mu\text{L}$  of the solution of NHS-biotin to the antibody (1 ml).

Incuber 1H at room temperature.

- 4- Dialyses the antibody against PBS+NaN<sub>3</sub> 0.01% (Use CelluSep membranes). The biotinylated antibody can be diluted to 1 mg/ml with 0.1% NaN<sub>3</sub> and 20% of glycerol for storage at  $-20^\circ\text{C}$  or  $+4^\circ\text{C}$ .

## Other Information

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

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

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### Literature

Carlsson, J., Drevin, H. and Axen, R. (1978). Protein thiolation and reversible protein-protein conjugation. N-succinimidyl-3-(2-pyridylthio)propionate, a new heterobifunctional reagent. *Biochem. J.* 173, 723-737.

Cumber A.J., Forrester J.A., Foxwell B.M., Ross W.C.J. and Thorpe P.E., Preparation of antibody-toxin conjugates. *Meth. Enzymol.* 1985, 112, 207-225

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Wang 1997, Rajur 1997, Gordon 1987

rev. B07E