

DSP Crosslinker Protocol and Product Information Sheet

Product Category: Homobifunctional Crosslinkers

Catalog Number(s): <u>c1106-100mg</u>, <u>c1106-1gm</u>, c1106-custom

Product Name: DSP Crosslinker

Alternative Name(s): DSP; DTSP; Lomant's Reagent; 3.3'-Dithiodipropionic acid di(N-

hydroxysuccinimide ester); Di(N-succinimidyl) 3,3'-dithiodipropionate; Dithiobis-

succinimidyl propionate

CAS Number: 57757-57-0 Chemical Formula: $C_{14}H_{16}N_2O_8S_2$ Molecular Weight: 404.42 Spacer Arm Length: 12.0 Å

General DSP Crosslinking Protocol

- 1. Prepare a 50 mM solution of DSP crosslinker, by dissolving 10mg DSP in 495 μ L of dry DMSO or dry DMF solvent.
- 2. Using a 20-fold molar excess approach (20:1 Crosslinker:Protein), add crosslinker solution to the protein sample in non-amine containing buffer (i.e. 25 mM Sodium Phosphate, pH 7.4), so that the final crosslinker concentration is between 0.5 to 5 mM. Optimal pH range is from 7 to 9.
- 3. Allow the sample to react at room temperature for 30-45 minutes. Allow slightly longer if sample must be kept on ice (recommended 2-3 hours). This reaction rate is not highly temperature sensitive.
- 4. Quench any unreacted DSP with 25 mM to 200 mM Tris, pH 7.4. Allow to react for 10-15 minutes at room temperature.
- 5. Desalt sample to remove unreacted DSP crosslinker (i.e. gel filtration, dialysis, etc.).

Intracellular DSP Crosslinking Protocol

- 1. Remove media by washing cells twice with non-amine containing buffer (i.e. 25 mM Sodium Phosphate, pH 7.4).
- 2. The crosslinking solution as noted in steps 1 and 2 above, then add the crosslinker solution to the cells in a final concentration of ~2 mM.
- 3. Incubate the reaction mixture for 30-45 minutes at room temperature or for 2-3 hours if on ice.
- 4. Quench any unreacted DSP with 25 mM to 200 mM Tris, pH 7.4. Allow quenching reaction to proceed for 10-15 minutes at room temperature.

Reference:

Wong, S.S. (1993) CRC Chemistry of Protein Conjugation and Crosslinking. CRC Press, Boca Raton, Florida.