

SPDP Crosslinker Protocol and Product Information Sheet

Product Category: Heterobifunctional Crosslinkers

Catalog Number(s): c1116-100mg, c1116-1g, c1116-custom

Product Name: SPDP Crosslinker

Alternative Name(s): 3-(2-Pyridyldithio)propionic acid N-hydroxysuccinimide ester

CAS Number: 68181-17-9 Chemical Formula: $C_{12}H_{10}N_2O_4S_2$

Molecular Weight: 312.36 Spacer Arm Length: 6.8 Å

Storage: Upon receipt store at -20°C (shipped at ambient temperature). Protect

from moisture (i.e. humidity); blanket under desiccated, inert gas.

General SPDP Protein Crosslinking Protocol

- 1. Allow vial of SPDP Crosslinker to fully equilibrate to ambient temperature before opening to prevent condensation inside the vial (SPDP is moisture-sensitive).
- Dissolve 5 mg of SPDP in 640 μL DMSO (cr8105-25ml) or DMF (cr8106-25ml) to give a 25 mM crosslinker solution.
- 3. Dissolve protein #1 (without active thiol residues) at a concentration of 1-5 mg/mL in 100 mM sodium phosphate buffer, pH 7.2 to pH 8.0, 1 mM EDTA.
- 4. Add 20 µL of 25 mM SPDP crosslinker solution to 1 mL of the above protein solution.
- 5. Allow reaction to proceed for 30-60 minutes at room temperature.
- 6. Remove unreacted SPDP crosslinker from protein containing solution through gel-filtration, such as Desalting Resin g4109-1gm (i.e. Sephadex® G-25).
- 7. Dissolve protein #2 (with active thiol residues) in 100 mM sodium phosphate pH 7.2 to 8.0, 1 mM EDTA buffer.
- 8. Add 0.2 to 1.0 molar equivalents of protein #2 solution to desalted activated protein #1.
- 9. Allow this reaction to proceed for 8 to 16 hours at room temperature.
- 10. To cleave the newly formed conjugate, incubate crosslinked product with 50 mM DTT (<u>cr8101-5x10mg</u>) for 90-120 minutes at room temperature or 1 hour at 45°C.

References:

Hermanson, G.T. 1996. Bioconjugates Techniques. Academic Press, San Diego, CA USA.