

Sulfo-SANPAH Crosslinker Protocol and Product Information Sheet

Product Category: Heterobifunctional Crosslinkers

Catalog Number(s): <u>c1111-100mg</u>, <u>c1111-1gm</u>, c1111-custom

Product Name: Sulfo-SANPAH Crosslinker

Alternative Name(s): N-Sulfosuccinimidyl-6-(4'-azido-2'-nitrophenylamino) hexanoate

CAS Number: 102568-43-4 Chemical Formula: $C_{16}H_{17}N_6NaO_9S$

Molecular Weight: 492.40 Spacer Arm Length: 18.2 Å

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

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

gas.

General Sulfo-SANPAH Crosslinking Protocol

- 1. Allow vial of Sulfo-SANPAH Crosslinker to fully equilibrate to ambient temperature before opening to prevent condensation inside the vial (Sulfo-SANPAH is moisture-sensitive and will hydrolyze).
- 2. Do not use amine or sulfhydryl containing buffers for the conjugation reaction. A suitable buffer is 25 mM Sodium Phosphate, 15 mM NaCl, pH 7.4.
- Immediately before use, prepare a 10 mM solution of Sulfo-SANPAH in water (Note: The Sulfo-NHS ester of Sulfo-SANPAH is susceptible to hydrolysis. If aliquoted into an aqueous solution and stored in it, then the Sulfo-SANPAH will degrade).
- 4. Using a 20-fold excess approach (20:1 Crosslinker:Protein), add Sulfo-SANPAH crosslinker solution to the protein sample so that the final crosslinker concentration is between 0.5 to 5 mM.
- 5. Allow the sample to react at room temperature for 45 minutes to 1 hour. Allow slightly longer if reaction must be done on ice (this reaction rate is only slightly slower at low temperatures).
- 6. Optional: Quench and unreacted Sulfo-SANPAH with 25 mM to 60 mM Tris and allow to react for 10-15 minutes at room temperature.
- 7. Desalt sample to remove unreacted Sulfo-SANPAH crosslinker (i.e. gel filtration, dialysis, etc.).
- 8. Photoactivate Sulfo-SANPAH with a high wattage UV lamp (wavelength between 300-460 nm). Carry out photolysis with lamp 5-10 cm directly above the reaction.

References:

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

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