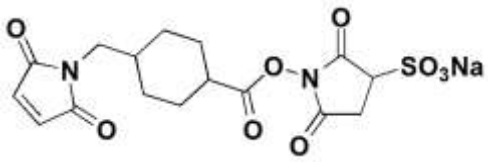
Sulfo-SMCC Protocol and Product Information Sheet

Product Category:	Heterobifunctional Crosslinkers
Catalog Number(s):	c1109-100mg, c1109-1gm, c1109-custom
Product Name:	Sulfo-SMCC Crosslinker
Alternative Name(s):	Sulfosuccinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate); 4-(N-Maleimidomethyl)cyclohexane-1-carboxylic acid-3-sulfo-N-hydroxysuccinimide ester
CAS Number:	92921-24-9
Chemical Formula:	$C_{16}H_{17}N_2O_9SNa$
Molecular Weight:	436.37
Spacer Arm Length:	8.3 Å
Storage:	Upon receipt store at -20°C or lower under desiccated, inert gas (shipped at ambient temperature). Protect from moisture (i.e. humidity).



Sulfo-SMCC Crosslinking Protocol

Note: The following protocol must be optimized empirically for each specific application. Typically, 10-50 molar excess of crosslinker to amine-containing protein yields sufficient maleimide activated protein for subsequent protein conjugation.

1. Allow vial of Sulfo-SMCC to fully equilibrate to ambient temperature before opening to prevent condensation inside the vial (Sulfo-SMCC is moisture-sensitive and will hydrolyze).
2. Avoid amine-containing buffers (i.e. Glycine, Tris, etc.) and sulfhydryls as they will compete with the desired conjugation reaction.
3. Prepare amine-containing protein sample in non-amine containing conjugation buffer (i.e. 0.1 M Sodium Phosphate, 0.15 M Sodium Chloride, pH 7.2).

   Note: To ensure best results, prepare reduced sulfhydryl-containing protein and make it ready for Step 8.
4. Immediately before use, prepare a 10mg/mL solution of Sulfo-SMCC by dissolving Sulfo-SMCC in deionized water (or 50 mM sodium phosphate).

   Note: Sulfo-SMCC should be dissolved in deionized water prior to adding it to a PBS solution. Sulfo-SMCC does not dissolve very well in buffers with > 0.05 M salt concentration, but once dissolved in water it can be further diluted in PBS (or other non-amine buffers). If Sulfo-SMCC does not completely dissolve, gentle warming of the solution in 40-50°C water bath for several minutes can aid in dissolving it.

   Note: The Sulfo-NHS ester of Sulfo-SMCC crosslinker is susceptible to hydrolysis. If aliquoted into an aqueous solution and stored in it, then the Sulfo-SMCC crosslinker will degrade.
5. Add sufficient Sulfo-SMCC stock solution to the protein solution to obtain 10-50 fold molar excess of crosslinking reagent over protein.

   Note: Alternatively, an amount of Sulfo-SMCC can be added to the protein solution required to give 10-50 fold molar excess. Dilute protein solutions (i.e. 1-2 mg/mL) may require increased molar excess of Sulfo-SMCC (i.e. $\geq$ 20 fold) to yield similar activation of a more concentrated protein solution (i.e. 10mg/mL) using 5 fold molar excess Sulfo-SMCC.
6. Using a 20-fold excess approach (20:1 Crosslinker:Protein), add Sulfo-SMCC crosslinker solution to the protein sample, so that the final crosslinker concentration is between 0.5 to 5.0 mM. Optimal pH range is from 7.0 to 7.5.

7. Allow reaction to proceed for 30-40 minutes at room temperature or ≥ 2 hours at 4°C. The reaction rate is not highly temperature sensitive.
8. Desalt activated protein sample to remove residual crosslinker protein through dialysis or gel filtration with a resin, such as Sephadex® G-25 (g4109) or equivalent, using conjugation buffer (i.e. 0.1 M Sodium Phosphate, 0.15 M Sodium Chloride, pH 7.2).
9. Add reduced Sulfhydryl-containing protein and desalted amine-containing protein in an appropriate molar ratio required for the final conjugate, and in accordance with the quantity of sulfhydryl and activated amines present between the two proteins.
10. React at room temperature for 30-40 minutes or ≥ 2 hours at 4°C.
11. Optional: Dialyze against 3 changes of PBS (100X the conjugate volume) to remove unconjugated proteins. Exchange buffer every 2 hours.

References:

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

Hermanson, G.T. (2008) Bioconjugate Techniques, 2nd Ed. Academic Press, New York.

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