

SMCC and Sulfo-SMCC

Cat.No.:C608317/C608318

C608317 package: 50/100 mg SMCC

C608318 package: 50/100 mg Sulfo-SMCC

Introductions

SMCC

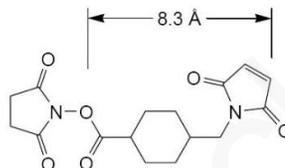
(succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate)

Molecular Weight: 334.32

Spacer Arm: 8.3 Å

Net Mass Added: 219.09

Storage: Upon receipt store desiccated at 4° C.Product is shipped at ambient temperature.



Sulfo-SMCC

(sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate)

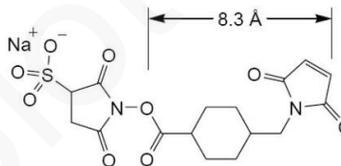
Molecular Weight: 436.37

Spacer Arm: 8.3 Å

Net Mass Added: 219.09

CAS #: 92921-24-9

Storage: Upon receipt store desiccated at -20° C.Product is shipped at ambient temperature.



SMCC and its water-soluble analog Sulfo-SMCC are heterobifunctional crosslinkers that contain N-hydroxysuccinimide (NHS) ester and maleimide groups that allow covalent conjugation of amine- and sulfhydryl-containing molecules. NHS esters react with primary amines at pH 7~9 to form amide bonds, while maleimides react with sulfhydryl groups at pH 6.5~7.5 to form stable thioether bonds. In aqueous solutions, NHS ester hydrolytic degradation is a competing reaction whose rate increases with pH. The maleimide group is more stable than the NHS-ester group but will slowly hydrolyze and loses its reaction specificity for sulfhydryls at pH values > 7.5. For these reasons, conjugations with these crosslinkers are usually performed at pH 7.2~7.5, with the NHS-ester (amine-targeted) reacted before or simultaneous with the maleimide (sulfhydryl-targeted) reaction.

The cyclohexane ring in the spacer arm of these reagents decreases the rate of hydrolysis of the maleimide group compared to similar reagents that do not contain this ring. This feature enables proteins that have been maleimide-activated with SMCC or Sulfo-SMCC to be lyophilized and stored for later conjugation to a sulfhydryl-containing molecule. Many maleimide-activated protein products are produced in this manner.

SMCC and Sulfo-SMCC are often used to prepare antibody-enzyme and hapten-carrier protein conjugates in a two-step reaction scheme. First, the amine-containing protein is reacted with a several-fold molar excess of the crosslinker, followed by removal of excess (nonreacted) reagent by desalting or dialysis; finally, the sulfhydryl-containing molecule is added to react with the maleimide groups already attached to the first protein.

Sulfo-SMCC is soluble in water and many other aqueous buffers to approximately 10 mM, although solubility decreases with increasing salt concentration. SMCC is not directly water-soluble and must be dissolved in an organic solvent such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF); subsequent dilution into aqueous reaction buffer is generally possible, and most protein reactants will remain soluble if the final concentration of organic solvent is less than 10%.

Procedure for Two-step Protein Crosslinking

Generally, a 10- to 50-fold molar excess of crosslinker over the amount of amine-containing protein results in sufficient maleimide activation to enable several sulfhydryl-containing proteins to be conjugated to each amine-containing protein. More dilute protein solutions require greater fold molar excess of reagent to achieve the same activation level. Empirical testing is necessary to determine optimal activation levels and final conjugation ratios for the intended application.

1. Material Preparation

- 1) Conjugation Buffer: phosphate-buffered saline (PBS = 100 mM sodium phosphate, 150 mM sodium chloride, pH 7.2) or other amine- and sulfhydryl-free buffer at pH 6.5~7.5 adding EDTA to 1~5 mM helps to chelate divalent metals, thereby reducing disulfide formation in the sulfhydryl-containing protein
- 2) Desalting column to separate modified protein from excess crosslinker and reaction byproducts (e.g., Spin Columns)
- 3) Amine-containing (Protein-NH₂) and sulfhydryl-containing proteins (Protein-SH) to be conjugated

2. Protocol

For best results, ensure that Protein-SH is prepared and ready to combine with Protein-NH₂ in step 5.

- 1) Prepare Protein-NH₂ in Conjugation Buffer.
- 2) Add the appropriate amount of crosslinker to the protein solution. The concentration of the Protein-NH₂ determines the crosslinker molar excess to use. Suggested crosslinker molar excesses are as follows
 - a. Protein samples < 1 mg/ml use 40-80-fold molar excess.
 - b. Protein samples of 1~4 mg/ml use 20-fold molar excess.
 - c. Protein samples of 5~10 mg/ml use 5- to 10-fold molar excess.

Table 1. Crosslinker preparation and molar excess to use for 1mL of sample. Immediately before use, dissolve crosslinker in the appropriate solvent at the concentration denoted in parentheses; then add the listed volume to a 1mL protein sample.

Protein-NH ₂ Concentration (based on a 50 kDa protein)	10 mg/mL	1 mg/mL	0.5 mg/mL
Crosslinker Molar Excess	5X	20X	50X
Sulfo-SMCC (in 50 mM sodium phosphate or water)	100 μ L (4.8 mg/mL)	40 μ L (4.8 mg/mL)	50 μ L (4.8 mg/mL)
SMCC (in DMSO or DMF)	100 μ L (3.7 mg/mL)	100 μ L (1.5 mg/mL)	100 μ L (1.8 mg/mL)

✓ If the Sulfo-SMCC solution does not completely dissolve, place the tube under hot running water or incubate for several minutes in a 50°C water bath.

3. Incubate reaction mixture for 30 minutes at room temperature or 2 hours at 4°C.
4. Remove excess crosslinker using a desalting column equilibrated with Conjugation Buffer.
5. Combine and mix Protein-SH and desalted Protein-NH₂ in a molar ratio corresponding to that desired for the final conjugate and consistent with the relative number of sulfhydryl and activated amines that exist on the two proteins.
6. Incubate the reaction mixture at room temperature for 30 minutes or 2 hours at 4°C.

✓ Generally, there is no harm in allowing the reaction to proceed for several hours or overnight, although usually the reaction will be complete in the specified time. To stop the conjugation reaction before completion, add buffer containing reduced cysteine at a concentration several times greater than the sulfhydryls of Protein-SH.

✓ Conjugation efficiency can be estimated by electrophoresis separation and subsequent protein staining.

Note

1. SMCC and Sulfo-SMCC are moisture-sensitive. Store reagent vial in desiccant. Equilibrate vial to room temperature before opening to avoid moisture condensation inside the container. Dissolve needed amount of reagent and use it immediately before hydrolysis occurs. Discard any unused reconstituted reagent. Do not store reagent in solution.
2. No-Weigh Microtube Handling: Immediately before use, puncture the microtube foil with a pipette tip, add 200 μ l of 50 mM sodium phosphate buffer (pH 7.0~7.5) or ultrapure water and pipette up and down to mix. After use, cut the used microtube from the microtube strip and discard. Store the unused microtubes in the foil pouch provided.

✓ Do not use phosphate-buffered saline (PBS) for initial dissolution of Sulfo-SMCC; the reagent does not dissolve well in buffers exceeding 50 mM total salts. However, once dissolved, the solution can be further diluted in PBS or other non-amine buffers.

3. Avoid buffers containing primary amines (e.g., Tris or glycine) and sulfhydryls during conjugation, because they will compete with the intended reaction. If necessary, dialyze or desalt samples into an appropriate buffer such as phosphatebuffered saline (PBS).
4. Molecules to be reacted with the maleimide moiety must have free (reduced) sulfhydryls. Reduce peptide disulfide bonds with Immobilized TCEP Disulfide Reducing Gel. For proteins, reduce disulfide bonds using 5 mM TCEP (1:100 dilution of TCEP Solution) for 30 minutes at room temperature, followed by two passes through a suitable desalting column (e.g., Desalt Spin Columns). Be aware that proteins (e.g., antibodies) may be inactivated by complete reduction of their disulfide bonds. Selective reduction of hinge-region disulfide bonds in IgG can be accomplished with 2-Mercaptoethylamine•HCl (2-MEA). Sulfhydryls can be added to molecules using N-succinimidyl S-acetylthioacetate (SATA) or 2-iminothiolane•HCl (Traut's Reagent), which modify primary amines.

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