SMCC Plus™ *Enhanced water solubility, crosslinking efficiency and stability*

Catalog number: 4503
Unit size: 5 mg

Component	Storage	Amount
SMCC Plus™ *Enhanced water solubility, crosslinking efficiency and stability*	Freeze (<-15 °C)	5 mg

OVERVIEW

Compared to SMCC and sulfo-SMCC, SMCC Plus has enhanced water solubility and crosslinking efficiency.

AT A GLANCE

Important It is important to store at <-15 °C and should be stored in cool, dark place.

It can be used within 12 months from the date of receipt.

Note SMCC Plus™ is soluble in water and many other aqueous buffers, but it is less stable than in DMSO or DMF. Thus, it is not recommended to make buffer stock solutions for repeated use. In addition, its solubility decreases with increasing salt concentration.

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare conjugation buffer for amine-containing protein, Protein-NH_2 (Buffer A): Phosphate buffered saline (PBS, pH 7.2) or other amine-free buffers with pH 7.2 to 8.0 are OK to use for this purpose.

   Note 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 PBS.

2. Prepare conjugation buffer for sulfhydryl-containing protein, Protein-SH (Buffer B): Sulfhydryl-free buffers at pH 6.5-7.5 are OK to use for this purpose.

   Note Avoid buffers containing sulfhydryls during conjugation because they will compete with the intended reaction. If necessary, dialyze or desalt samples into an appropriate buffer such as PBS.

   Note The addition of 1-5 mM EDTA helps to chelate divalent metals, thereby reducing disulfide formation in the sulfhydryl-containing protein.

3. Prepare desalting column to separate modified protein from excess crosslinker and reaction by-products.

4. Prepare amine-containing (Protein-NH_2) and sulfhydryl-containing protein (Protein-SH) solutions in their Conjugation Buffers A and B.

5. Prepare Protein #1-SMCC Plus™ conjugate: Add the appropriate amount of SMCC Plus™ to Protein #1 solution in buffer A. The concentration of the Protein-NH_2 determines the molar ratio of SMCC Plus™ required. The required amount of SMCC Plus™ is suggested as follows, but the best ratio has to be determined experimentally by using the target proteins:

- Protein samples < 1 mg/mL use 40-80-fold molar excess.
- Protein samples of 1-4 mg/mL use 20-fold molar excess.
- Protein samples of 5-10 mg/mL use 5- to 10-fold molar excess.

6. Incubate the above reaction mixture at room temperature for 30 - 60 minutes or at 4 °C for 2 - 4 hours.

   Note To stop the conjugation reaction before completion, add buffer containing reduced cysteine at a concentration several times greater than the sulfhydryls of Protein-SH.

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

7. Remove excess SMCC Plus™ using a desalting column equilibrated with Conjugation Buffer A.

8. Combine and mix sulfhydryl-containing (Protein-SH) and desalted amine-containing protein (Protein-NH_2)-SMCC Plus™ conjugate 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.

   Note Molecules to be reacted with the maleimide moiety must have free (reduced) sulfhydryls. For proteins, reduce disulfide bonds using 5 mM TCEP at room temperature for 30 minutes, followed by two passes through a suitable desalting column. 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 may be added to molecules using N-succinimidyl S-acetylthioacetate (SATA) or 2-iminothiolane•HCl (Traut's Reagent), which modify primary amines.

Storage Conditions

Store at 2 °C to 8 °C. Expiration date is one year from the date of receipt.

Note SMCC Plus™ is moisture-sensitive. Store desiccated. Equilibrate vial to room temperature before opening to avoid moisture condensation. Dissolve needed amount of reagent and use it immediately before hydrolysis occurs. Discard any unused reconstituted reagent. Do not store reagent in solution.

EXAMPLE DATA ANALYSIS AND FIGURES

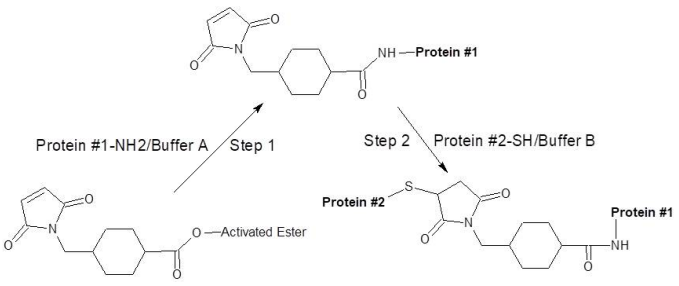

Figure 1. Overview of procedure for Teo-steps Protein Cross-linking

DISCLAIMER

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