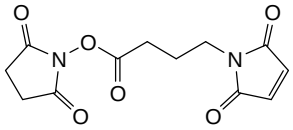
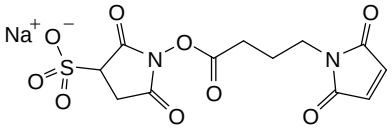
INSTRUCTIONS

GMBS and Sulfo-GMBS

22309 22324 1763.0

Number	Description
22309	**GMBS** (*N*-[γ-maleimidobutyryloxy]succinimide ester), 50 mg Molecular Weight: 280.23 Spacer Arm Length: 7.3 Å CAS #: 80307-12-6 **Storage:** Upon receipt store desiccated at 4° C. Product is shipped at ambient temperature.
22324	**Sulfo-GMBS** (*N*-[γ-maleimidobutyryloxy]sulfosuccinimide ester), 50 mg Molecular Weight: 382.28 Spacer Arm Length: 7.3 Å **Storage:** Upon receipt store desiccated at -20° C. Product is shipped at ambient temperature.





Introduction

GMBS and its water-soluble analog Sulfo-GMBS are heterobifunctional cross-linkers 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 the maleimides react with sulfhydryl groups at pH 6.5-7.5 to form stable thioether bonds. In aqueous solutions, hydrolytic degradation of the NHS ester 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 also lose its reaction specificity for sulfhydryls at pH values > 7.5. For these reasons, conjugation experiments involving these cross-linkers are usually performed at pH 7.2-7.5, with the NHS-ester (amine-targeted) reaction being accomplished before or simultaneous with the maleimide (sulfhydryl-targeted) reaction.

GMBS and Sulfo-GMBS 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 cross-linker, 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-GMBS is soluble in water and many other aqueous buffers to approximately 10 mM, although solubility decreases with increasing salt concentration. GMBS is not directly water-soluble and must be dissolved first 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%.

Important Product Information

- GMBS and Sulfo-GMBS cross-linkers are moisture-sensitive. Store vial of reagent in desiccant at the specified temperature. 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 attempt to make and store stock solutions.
- 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 phosphate buffered saline (PBS).

- Molecules to be reacted with the maleimide moiety must have free (reduced) sulfhydryls. Reduce peptide disulfide bonds with Immobilized TCEP Disulfide Reducing Gel (Product No. 77712). Reduce disulfide bonds in high molecular weight proteins using 5 mM TCEP (1:100 dilution of Bond-Breaker® TCEP Solution, Product No. 77720) for 30 minutes at room temperature, followed by two passes through an appropriate desalting column (e.g., Zeba™ Desalt Spin Columns). Be aware that proteins (e.g., antibodies) may be inactivated by complete reduction of disulfide bonds they contain. Selective reduction of hinge-region disulfide bonds in IgG may be accomplished with 2-Mercaptoethylamine•HCl (2-MEA, Product No. 20408). Sulfhydryls may be added to molecules using *N*-succinimidyl *S*-acetylthioacetate (SATA, Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101), which modify primary amines.

Procedure for Two-step Protein Cross-linking

Generally, a 10- to 50-fold molar excess of cross-linker 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 level of activation. Empirical testing is necessary to determine activation levels and final conjugation ratios that are optimal for the intended application.

A. Material Preparation

- Conjugation Buffer: Phosphate buffered saline (PBS, pH 7.2; e.g., Product No. 28372) or other amine- and sulfhydryl-free buffer at pH 6.5-7.5 (see Important Product Information) – adding EDTA to 1-5 mM helps to chelate divalent metals, thereby preventing disulfide formation in the sulfhydryl-containing protein
- Desalting column to separate modified protein from excess cross-linker and reaction byproducts (e.g., Zeba™ Desalt Spin Columns)
- Amine-containing protein (Protein-NH_2) and sulfhydryl-containing protein (Protein-SH) to be conjugated

B. Protocol

Note: For best results, ensure that Protein-SH is prepared (see Important Product Information) and ready to combine with Protein-NH_2 in step 5.

1. Dissolve Protein-NH_2 in Conjugation Buffer at 0.1 mM (e.g., 5 mg in 1 ml for a 50 kDa protein).
2. Add cross-linker to dissolved Protein-NH_2 at 1 mM final (= 10-fold molar excess):
   - For Sulfo-GMBS, add 0.382 mg per milliliter of Protein-NH_2 solution or dissolve 3.82 mg Sulfo-GMBS in 1 ml Conjugation Buffer (makes 10 mM temporary stock) and immediately add 100 µl/ml of Protein-NH_2 solution.
   - For GMBS, dissolve 2.80 mg GMBS in 1 ml DMSO (makes 10 mM) and then add 100 µl/ml of Protein-NH_2 solution.
3. Incubate reaction mixture for 30 minutes at room temperature or 2 hours at 4°C.
4. Remove excess cross-linker using a desalting column equilibrated with Conjugation Buffer.

   Note: Follow the desalting column product instructions to determine which fractions contain Protein-NH_2. Alternatively, locate the protein by measuring for fractions having peak absorbance at 280 nm; however, be aware that the NHS-ester leaving group also absorbs strongly at 280 nm.
5. Combine and mix Protein-SH and desalted Protein-NH_2 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.

   Note: 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.

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

Related Thermo Scientific Products

Table 1. Noncleavable NHS/Maleimide cross-linkers.

Cross-linker Name	Spacer Arm Length (Å)	Spacer Arm Composition (between ester and maleimide)	Product No. (NHS)	Product No. (Sulfo-NHS)
AMAS	4.4	Alkane	22295	NA
BMPS	5.9	Alkane	22298	NA
GMBS	7.3	Alkane	22309	22324
MBS	7.3	Aromatic	22311	22312
SMCC	8.3	Cyclohexane	22360	22322
EMCS	9.4	Alkane	22308	22307
SMPB	11.6	Alkane/Aromatic	22416	22317
SMPH	14.2	Alkane/Amide	22363	NA
LC-SMCC	16.2	Alkane/Amide/Cyclohexane	22362	NA
KMUS	16.3	Alkane	NA	21111

Product References

1. Kitagawa, J., *et al.* (1981). Preparation and characterization of hetero-bifunctional cross-linking reagents for protein modifications. *Chem. Pharm. Bull.* **28:** 1130-5.
2. Tanimori, H., *et al.* (1981). Enzyme immunoassay of neocarzinostatin using ß-galactosidease as label. *J. Pharmacobiodyn.* **4:** 812-9.
3. Fujiwara, K., *et al.* (1988). Sandwich enzyme immunoassay of tumor-associated antigen sialosylated Lewisx using ß-D-galactosidase coupled to a monoclonal antibody of IgM isotype. *J. Immunol. Meth.* **112:** 77-83.
4. Adessi, C., *et al.* (2000). Solid phase DNA amplification: characterization of primer attachment and amplification mechanisms. *Nucl. Acid Res.* **28(20):**e87.
5. Brinker, A., *et al.* (2002). Ligand discrimination by TPR domains. *J. Biol. Chem.* **277(22):** 19265-75.
6. de Haan, L., *et al.* (2002). Enhanced delivery of exogenous peptides into the class I antigen processing and presentation pathway. *Infec. Immunity* **70(6):** 3249-58.
7. Geller, B.L., *et al.* (2003). Inhibition of gene expression in Escherichia coli by antisense phosphorodiamidate morpholino oligomers. *Antimicrob. Agents Chemother.* **47(10):** 3233-9.
8. Guillaume, P., *et al.* (2003). Soluble major histocompatibility complex-peptide octamers with impaired CD8 binding selectively induce fas-dependent apoptosis. *J. Biol. Chem.* **278(7):** 4500-9.