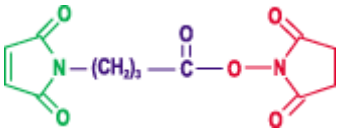
FT-49608A

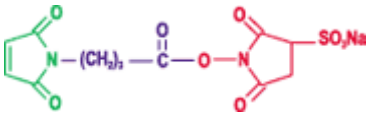
MPS, GMBS, EMCS, KMUS
Heterobifunctionnal crosslinkers

Products description

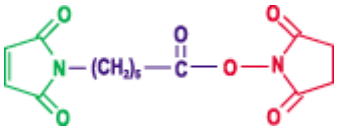
Catalog number: UP49608A, 100mg UP49608B, 50mg
Name: **GMBS**
Formula : MaleimidoButyryloxy-Succinimide ester
Spacer 7.3 Å
MW= 280.24 ; CAS : 80307-12-6



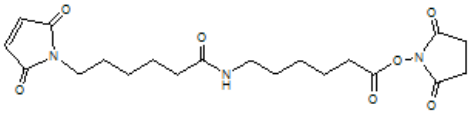
Catalog number: UP96999A, 100mg
Name: **Sulfo-GMBS**
Formula : m-MaleimidoButyryloxy-SulfoSuccinimide ester
MW= 382.28; CAS: 103848-61-9



Catalog number: UP19548A, 100mg UP19548B, 50mg
Name: **EMCS** (HMCS)
Formula : N-(e-MaleimidoCaproyloxy)-N-HydroxySuccinimide ester
Spacer 9.4Å
MW= 308.29; CAS: 55750-63-5

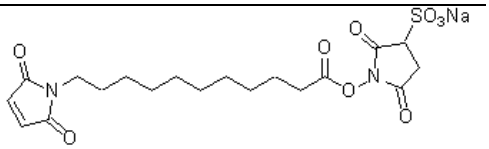


Catalog number: BI1221, 50mg
Name: **Sulfo-EMCS (LC-EMCS)**
Formula : N-(e-MaleimidoCaproyloxy)SulfoSuccinimide ester
MW= 421.45

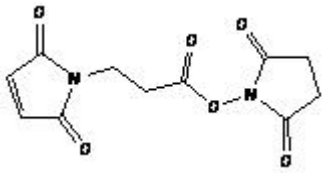


Catalog number: T31950, 50mg
Name: **KMUS**
Formula : N-(e-MaleimidoCaproyloxy)-N-HydroxySuccinimide ester
Spacer Å16.3
MW= 308.29.

Catalog number: L7712A, 50mg
Name: **Sulfo-KMUS**
Formula : Sulfo-Succinimidyl 11-maleimidodoundecanoate; N-[k-Maleimidoundecanoyloxy]sulfosuccinimide ester
MW= 480.47, CAS: 211236-68-9



Catalog number: L7726B, 250mg
Name: **MPS**
Formula :
Spacer 6.9 Å (2 carbons)
MW= 266.21
CAS:



Storage : +4°C, protect from moisture and light (L)
-20°C (sulfonated derivatives) , protect from moisture and light (M)

General Considerations

Cross-linkers are chemical reagents used to conjugate molecules together by a covalent bound. Several atoms separate the 2 molecules, forming the 'spacer arm'. The conjugate associates the characteristics and biological activities of each component.

Cross-linkers have become important tools for the preparation of conjugates used in a lot of immunotechnologies, and for protein studies (structure, interactions, activity, degradation…). To that point, heterobifunctionnal cross-linkers are probably the most interesting, because they present 2 reactivities that allow the conjugation of molecules in a defined manner, avoiding notably the formation of dimeres and polymeres. The choice of reactivities is determinant to the design of the right conjugate. Considering the final result, an important other thing is the nature and length of the spacer. The cross-linkers contain the 2 reactivities toward amines, through the succinimide group, and a reactivity toward sulfhydryls, through the maleimide group.

Uptima offers a high quality cross-linkers to answer the needs of coupling proteins and peptides for biologogical and immunoassays like (other cross-linkers are available):

- Obtention of immunogens carrier-hapten
- Obtention of labeled affine probes: for example, antibodies coupled to enzyme for immunoblotting, fluorophore-peptides conjugates for the study of receptors, enzyme-drugs for using as tracers in ELISA…
- Obtention of oligomeric conjugates : conjugates of oriented peptides for immunization, dimeric proteins for structural studies, graffting haptens onto cells…
- Obtention of biologically active conjugates: specific antibody coupled to drugs for immunotargetting techniques, immunotoxins, …

Scientific and Technical Information

- The chemical group **N-hydroxysuccinimydyl (NHS)** reacts in aqueous phase on primary ($-NH_2$) and secondary amines (=NH) (in fact on its deprotonated form), at pH 7-9.5, and optimally at in a pH ~8.5. The reaction is very specific, targeting typically ε-amines present in Lys aminoacid in any proteins, but in a lower proportion on terminal α-NH_2 of peptidic chains. The reaction competes with hydrolysis, which increases with pH and with the high dilutions of the molecule that should be derivatized.
- The **sulfonyl moeity (NaSO3)** introduces a hydrophilic group, that allows the product not to cross biological membrans. This is particularly useful to modify, in situ on cells, proteins presented outside membranes, and if one wants to avoid the modification of intracellular proteins that may affect further analysis. An other interest of the sulfonyl group is to permit the solubilization of the product directly in aqueous buffers, up to 10mM, avoiding the use of organic solvants like DMSO or DMF, that are possibly nocive to cells or applications. An alterantive approach is to use a homologous crosslinker with a hydrophilic spacer such as MAL-PEO-NH #AL6580.
- The **spacer arm** of different cross-linkers differ by the length. Spacers of other nature are also offered (ask). Sulfo-KMUS is soluble in water and many other aqueous buffers to approximately 10 mM, although solubility decreases with increasing salt concentration. More concentrated solutions of Sulfo-KMUS can be prepared 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%.
- The **maleimide group** reacts very specifically with sulfhydryls –SH at neutral pH 6.5-7.5. The reaction is rapid (a few minutes for cystein), but in the absence of –SH, it is well stable. The hydrolysis forming maleimic acid becomes noticeable when pH go up 8.0, where the reactivity with amines begins to be possible. In usual conditions, one should start with a ratio of 10-20 moles of maleimide per mole of protein. With SH-peptides, a molar 1:1 incubation ratio allows almost 1:1 coupling.
- The molecules to be reacted with the maleimide moiety must have free (reduced) sulfhydryls. Reduce disulfide bonds using 5 mM TCEP (#UP242214) for 30 minutes at room temperature, followed by an appropriate desalting step (for peptide you also can use Immobilized TCEP Disulfide Reducing Gel #FN0780). 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-MEA, #BI1191. Sulfhydryls may be added to molecules using SATA, #UP84235A or Traut's Reagent, #UP42425A, which modify primary amines.

Directions for Use

Handling

Allow the vial to reach room temperaure before opening.

Material Preparation

- Conjugation Buffer: Phosphate buffered saline (PBS, pH 7.2; e.g., Product No. UP68723A) 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 preventing disulfide formation in the sulfhydryl-containing protein
  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).
- Several accessory reagents are proposed in the protocol, and can be found at http://www.interchim.com/interchim/customers/articles.cfm

Protocol 1 : Conjugating an antibody with an enzyme, Peroxidase or Alkaline Phosphatase

This standard protocol can be applied to polyclonal and monoclonal purified antibodies.

- Dialyse the antibody at 10 mg/ml in PBS (NaCl 150mM, phosphate 20mM pH7.5) 4mM EDTA
- Add 10mM of DTT (#UP284250) or TCEP (#UP242210), incubate 1H at +37°C
- Desalt the antibody by gelfiltration with disgazed PBS buffer to elute. The dessalted antibody can be monitored in eluted fractions by measuring absorbance at 280nm, or a protein assay. SH concentration can be dosed by the DTNB (#UP01566) method. Use the antibody rapidly because SH oxidizes easily in contact of air; or else, keep it at +4°C if possible under nitrogen.
- Dialyse the enzyme at 10mg/ml in PBS. The buffer should be free of amines (no Tris)
- Add 3 mg * of cross-linker per ml of enzyme while mixing, and incubate for 15min at +37°C. Protect from light.

Note: *: the quantity of cross-linker to protein should be determined depending on cross-linker and protein molecular weight, of the desired degree of derivatization, and of reaction conditions. It is usually between 5 and 20 mol /mol of protein)

Note: non sulfonated crosslinkers should be added as a DMSO solution

- Desalt the maleimide activated enzyme by gel filtration in PBS. Fractions containing the enzyme can be identified by absorbance measurement at 280nm, or any other means (Coo Assay #UPF8640A, addition of substrate). Use this activated enzyme rapidly.
- Add the reduced antibody to the activated enzymes, and incubate for 30min at room temperature, protected from light.
- Desalt the conjugate by gel filtration in PBS (peroxidase) or TBS (Tris 10mM NaCl 150mM pH7.4, 1mM MgCl2) for the alkaline phosphatase.
- Store the conjugate at +4°C with preservatives and 20% glycerol.

The immuno-conjugate can be titrated by ELISA on a coating of relevant antigen that is recognized by the antibody, and with a suitable substrate (pNPP #UP664790 for the alkaline phosphatase; TMB #UP664780 for the peroxidase).

This protocole can be adapted to other proteins than antibodies and enzymes. A set up is generally necessary for each application. It is important to check that the molecules to be coupled are pure enough. One should contain amines, the other sulfhydryls. Sulfhydryls are rarely naturally present, but generated either by reduction like in the protocole 1, or by chemical modification of amines with SATA #UP84235A, or Iminothiolane #UP42425A reagent.

Protocol 2: Conjugating a Cys-peptide to a protein (antibodies, carrier…)

Peptides are frequently synthetized with a terminal cystein in terminal positions, to facilitate their attachment to other molecules. One can adapt the protocole 1 by substituting the antibody for the peptide and the enzyme for the protein.

Rem : The cysteine (Cys-SH) of lyophilized peptides oxydizes readily to the air, forming dimeric peptides (with disulfide bridges –S-S-), and impairing the right conjugation. The concentration of –SH can be quantified by the DTNB (UP01566H) method. If the –SH level was sufficient, the reduction then dessalting steps are naturally not useful.

Rem : Uptima offers optimised carriers, MaxiBind™ to prepare peptides-conjugates for immunization and screening purposes. Ask for them!

Procedure 3 - two-step Protein Cross-linking using KMUS

Note: Amine-containing protein (Protein-NH2) and sulfhydryl-containing protein (Protein-SH) should be prepared in suitable buffer (devoid amine or sulfhydryl additives; see technical info). Notably protein-SH should be ready to combine for step 5 (not oxidized).

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. To dissolved Protein-NH_2, add cross-linker at 1 mM final (0.480 mg of Sulfo-KMUS per milliliter). Alternatively, dissolve 4.80 mg Sulfo-KMUS in 1 ml of DMF or DMSO (makes 10 mM temporary stock) and immediately add 100 µl of the stock solution to 1 ml of Protein-NH_2 solution.

Note: Generally, a 10- to 50-fold molar excess of cross-linker over the amount of amine-containing protein enable several sulfhydryl-containing proteins to be conjugated to each amine-containing protein. More dilute protein solutions require greater fold molar excess of reagent. However, it is recommended to calibrate the final conjugation ratios (test a set of crosslinker / proteins) for optimal results in your application.

3. Incubate reaction mixture for 30 minutes at room temperature or 2 hours at 4°C.

4. Remove excess cross-linker using a dialysis or desalting column equilibrated with Conjugation Buffer.

Note: Desalting by dialysis (use CelluSep tubings) is easy and hands-off and economic. The use of gelfiltration desalting column (UP848742) is quicker, but needs to determine which fractions contain Protein-NH2 (i.e. by measuring for absorbance at 280 nm; note that the NHS-ester leaving group also absorbs strongly at 280 nm: collect excluded fractions only! a protein assay is also required to asses the yield of collected proteins).

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: the reaction is usually complete in the specified time, and can proceed for several hours or overnight without harm. It can eventually be stopped by adding buffer containing reduced cysteine in the sulfhydryls of Protein-SH.

It is usually needed to desalt conjugate the conjugate for downstream uses. This can be performed as above/step 4.
Conjugation efficiency may be estimated by electrophoresis separation and subsequent protein staining.

References – Sulfo-EMCS

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- **Fens M.** *et al.*, Angiogenic endothelium shows lactadherin-dependent phagocytosis of aged erythrocytes and apoptotic cells, *Blood*, 111: 4542 - 4550 (2008) Abstract

References - KMUS

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2) **Anjaneyulu**, **P. S. R.** *et al.*, "Reactions of N-Hydroxysulfosuccinimide Active Esters", *Int. J. Peptide Protein Res.*, 1987, 30, 117.
3) **Fukami Y.** *et al.*, "Evidence for Autoinhibitory Regulation of the c-src Gene Product a Possible Interaction Between the Src Homology 2 Domain and Autophosphorylation Site", *J. Biol. Chem.*, 1993, 268, 1132.
4) **Weber A**. *et al.*, Ligand-Receptor and Receptor-Receptor Interactions Act in Concert to Activate Signaling in the *Drosophila* Toll Pathway, *J. Biol. Chem.*, Vol. 280, Issue 24, 22793-22799 (2005) Article
5) **DeCory T**. *et al.*, Development of an Immunomagnetic Bead-Immunoliposome Fluorescence Assay for Rapid Detection of *Escherichia coli* O157:H7 in Aqueous Samples and Comparison of the Assay with a Standard Microbiological Method, *Applied and Environmental Microbiology*, p. 1856-1864, Vol. 71, No. 4 (2005) Article
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Other information

For use *in vitro* only, not for diagnostic.

Catalog size quantities and prices may be found at http://www.interchim.com

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