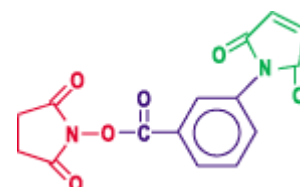


MBS, sMBS, SMPB, sSMPB

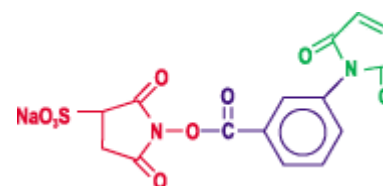
Heterobifunctional cross-linkers

Product Description

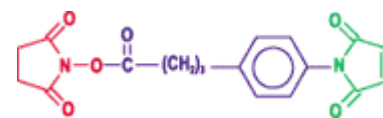
Catalog number: UP21608A, 100mg UP21608B, 50mg
Name: **MBS**
Formula: m-MaleimidoBenzoyl -N-hydroxySuccinimidyl ester
 $C_{15}H_{10}N_2O_6$, **M.W.= 314.26**, CAS[58626-38-3]



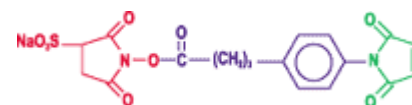
Catalog number: UP52444A, 100mg UP52444B, 50mg
Name: **Sulfo-MBS**
Formula: m-MaleimidoBenzoyl -SulfoSuccinimidyl ester
 $C_{15}H_9N_2O_9SNa$, **M.W.= 416.3**



Catalog number: UP28072A, 100mg UP28072B, 50mg
Name: **SMPB**
Formula: Succinimidyl 4-(p-MaleimidoPhenyl)-Butyrate
 $C_{18}H_{16}N_2O_6$, **M.W.= 356.34**, CAS[79886-55-8]



Catalog number: UP52757A, 100mg UP52757B, 50mg
Name: **Sulfo-SMPB**
Formula: SulfoSuccinimidyl -4-(p-maleimidophenyl)-butyrate
 $C_{18}H_{15}N_2O_9SNa$, **M.W.= 458.38**



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 bond. 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, heterobifunctional 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 dimers and polymers. 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 biological 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, grafting haptens onto cells...
- Obtention of biologically active conjugates: specific antibody coupled to drugs for immunotargetting techniques, immunotoxins, ...

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Scientific and Technical Information

- The chemical group N-hydroxysuccinimide (NHS) reacts in aqueous phase on primary ($-NH_2$) and secondary amines ($=NH$) (in fact on its deprotonated form), optimally at neutral pH or higher : amines present in proteins (Lys aminoacid) and in a lower proportion on NH_2 located in terminal peptidic chains. The reaction competes with hydrolysis, that increases with pH, and with the high dilutions of the molecule that should be derivatized.
- The sulfonyl moiety ($NaSO_3$) introduces a hydrophilic group, that allows the product not to cross biological membranes. 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 solvents like DMSO or DMF, that are possibly nocive to cells or applications.
- The spacer arm of different cross-linkers differ by the length. Spacers of other nature are also offered (ask).
- The maleimide group reacts very specifically with sulfhydryls $-SH$ at neutral pH 6.5-7.5, in comparison to iodoacetamides which react also with tyr, his, met. The reaction is rapid (a few minutes for cystein), but in the absence of $-SH$, maleimide stay well stable. 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. note: Hydrolysis forming maleimic acid may compete significantly when pH go up 8.0, where the reactivity with amines begins to be possible. Then pH higher than 8.5 could affect conjugate stability, or a nucleophilic reaction with an adjacent amine can ring-open to yield crosslinked products.

Use

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

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

- 1- Dialyse the antibody at 10 mg/ml in PBS (NaCl 150mM, phosphate 20mM pH7.5) 4mM EDTA
- 2- Add 10mM of DTT (#UP284250) or TCEP (#UP242210), incubate 1H at +37°C
- 3- Desalt the antibody by gel filtration with degassed PBS buffer to elute. The desalted 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.
- 4- Dialyse the enzyme at 10mg/ml in PBS. The buffer should be free of amines (no Tris)
- 5- 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
- 6- 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.
- 7- Add the reduced antibody to the activated enzymes, and incubate for 30min at room temperature, protected from light.
- 8- Desalt the conjugate by gel filtration in PBS (peroxidase) or TBS (Tris 10mM NaCl 150mM pH7.4, 1mM $MgCl_2$) for the alkaline phosphatase.
- 9- 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.

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FT-UP21608

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

Peptides are frequently synthesized with a terminal cysteine 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!

Other Information

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

For any information, please contact Uptima, or your local distributor.

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Literature

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