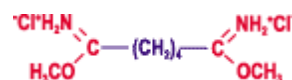


DMA, DMP, DMS

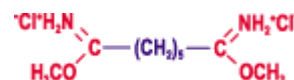
Homobifunctional imidoester cross-linkers

Product Description

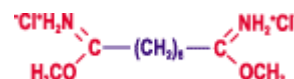
Catalog number: UP09962A, 1g UP099628B, 10g
 Name: **DMA (DMAI)**
 Formula : Dimethyl Adipimidate.2HCl
 $C_8H_{18}N_2O_2Cl_2$, M.W.= 245.14, CAS[14620-72-5]



Catalog number: UP362009, 100mg UP36200A, 1g UP36200B, 10g
 Name: **DMP (DMPI)**
 Formula : Dimethyl Pimelimidate.2HCl
 $C_9H_{20}N_2O_2Cl_2$, M.W.= 259.18, CAS[58537-94-3]



Catalog number: UP06633A, 1g UP06633B, 10g
 Name: **DMS (DMSI)**
 Formula : Dimethyl Suberimidate.2HCl
 $C_{10}H_{22}N_2O_2Cl_2$, M.W.= 273.21, CAS[34490-86-3]



Storage : +4°C (possible at -20°C), protect from moisture and light (L)
 Stable on year

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...). Homobifunctional cross-linkers present 2 identical reactivities. This sheet describes DMA, DMP, and DMS that are reactive toward amines, through the imidoester group, and DTPB that contains a cleavable spacer, allowing the conjugate to be easily broken under defined conditions.

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 oligomeric conjugates : conjugates of oriented peptides for immunization, dimeric proteins for structural studies, grafting haptens onto cells...
- Reticulation of proteins onto supports (polystyrene, nitrocellulose, agarose...): i.e. DMP easily grafts antibodies onto protein A – agarose.
- Crosslinking of complexes for structure and binding studies
- Obtention of labeled affine probes and biologically active conjugates

Scientific and Technical Information

- Imido ester react with primary amine groups to form at pH7-10 imido amides bound, without affecting the ionic charge of proteins.
- The reaction is specific to amines at pH7-9, but above pH9.5, a reaction may occur with epsilon-amines

Contact your local distributor

Uptima, powered by



213 Avenue J.F. Kennedy - BP 1140
 93163 Montesson Cedex - France
 Tél. 04 70 93 88 55 - Fax 04 70 93 82 86

Uptima@interchim.com

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- These cross-linkers are water soluble and membrane permeable.
- Unlike other amine reactive groups (i.e. succinimidyl group), it bears effectively a positive charge (at physiological pH) that replaces amine charge. This helps preserving the biological activity of several conjugates, even after extensive reaction (Wong 1991).
- The formed imido amide bond is stable at neutral pH, but slowly hydrolyses at high pHs. It can be broken by ammonium hydroxide reaction, regenerating the original amine group.
- The spacer arm of different cross-linkers differ by the length, providing tools for the study of protein to protein interactions, (Pepsinky 1980) and of oligomeres vicinity. The thiol-cleavable spacer of DTPB is useful for purification needs, releasing one ligand when the other has been immobilized thought an antibody (via Protein A #IPA300) or a suitable gel (Ami.R.Gel #56408).
- Solubility is >50mg (DMA, DMS)/ml in water, and 5%(DMS) in methanol

Guidelines for Use

Protocol 1 : Conjugating proteins and peptides

This protocol can be adapted to each proteins. A set up is generally necessary for each application. It is important to check that the molecules to be coupled are pure enough.

- 1- Prepare protein or peptide at 1-5 mg/ml in 0.2 M triethanolamine pH8.0.
Other buffer may be used provided pH is 7-9 and do not contains amines (no Tris), for example phosphate, carbonate, MOPS and Hepes.
Buffer exchange may be performed by gelfiltration, dialysis, or simply dissolving lyophilized material.
Don't store and use later the cross-linker solution.
- 2- Weight out the desired quantity of cross-linker and make eventually a 100 mM solution.
Allow the vial to reach the room temperature before opening. Protect remaining powder from moisture.
- 3- Add 10-30 fold molar excess of cross-linker to protein solution. Incubate for 1 hour at +4°C under agitation.
Incubation may be performed also at room temperature, but higher temperature should be avoided.
- 4- Block the reaction by adding acetic acid (100 mM) or Tris or glycine (100 mM pH 7.5) to quench for 1 hour.
- 5- Desalt the conjugate by gel filtration in PBS (peroxidase) or TBS (Tris 10 mM NaCl 150 mM pH7.4, 1 mM MgCl₂) for the alkaline phosphatase.

Protocol 2: Coupling Antibodies to Protein A or G beads

use 2 mg of antibody per ml wet beads (use appropriate antibody/protein A or G combination)

- 1- mix antibodies with beads and bind at room temperature for at least 1 hr (on roller)
- 2- wash the beads twice with 10 volumes borate buffer (0.2 M Na-borate pH 9.0), spin each time 3 min at 4000 rpm
- 3- resuspend beads in 10 volumes borate buffer ; remove equivalent of 10 µl beads (= before sample)
- 4- add solid DMP (dimethylpimelimidate) to a final concentration of 20 mM [52 mg for 10 ml]
- 5- mix on roller for 30 min at room temperature ; remove equivalent of 10 µl beads (= after sample)
- 6- stop reaction by washing the beads twice in 0.2 M ethanolamine pH 8.0
- 7- incubate on roller for 2 hr at room temperature in 0.2 M ethanolamine pH 8.0
- 8- wash beads twice with PBS; beads can be stored in PBSs at +4 °C ; check coupling by analysing the before and the after sample on a 10 % SDS gel or other mean

Other information

For use *in vitro* only, not for diagnostic. For any information, please contact Uptima, or your local distributor.

Literature

- DMAI:** Hartman, F.C. and Wold, F. (1967). Cross-linking of bovine pancreatic ribonuclease A with dimethyl adipimidate. *Biochem.* 6(8), 2439-2448.
- DMPi:** Schneider, C., Newman, R.A., Sutherland, D.R., Asser, U. and Treaves, M.F. (1982). A one-step purification of membrane proteins using a high efficiency immunomatrix. *J. Biol. Chem.* 257(18), 10766-10769.
- Wong S.S., *Chemistry of Protein Conjugation and Crosslinking*, CRC Press Publishers, Boca Raton, 1991
- Pepsinky R.B., Capiello D., Wilkowski C., and Vogt V.M., *Chemical Crosslinking of Proteins in Avian Sarcoma and Leukemia Viruses*, *Virology* 1980, 102, 205-210

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DMSI: Wang, D. and Moore, S. (1977). Polyspermine-ribonuclease prepared by cross-linkage with dimethyl suberimidate. *Biochem.* 16(13), 2937-2941.

Schneider C, Newman R.A., Sutherland D.R., Asser U. and Greaves M.F., One step Purification of Membrane Proteins Using a High Efficiency Immunomatrix, *J.Biol.Chem.* 1982, 257, 10766-10769

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CelluSep™: dialysis membranes

Other cleavable homobifunctional amine reactive crosslinkers: DSP [UP18971](#) and DTPB [UP99796](#) (thiol cleavable), DST [UP28068](#) (oxidizer cleavable), EGS [UP280067](#) (mild alkaline cleavable)...

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