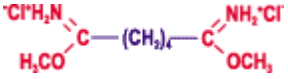
FT-06633A

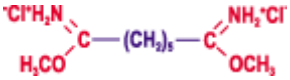
DMA, DMP, DMS
Homobifunctional imidoester cross-linkers

Products Description

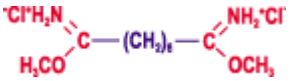
Catalog number: UP09962A, 1g UP099628B, 10g
Name: **DMA (DMAI)**
Formula : Dimethyl Adipimidate.2HCl
CAS[14620-72-5], M.W.= **245.14**



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



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



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

Scientific and Technical Information

Cross-linkers are chemical reagents used to conjugate molecules together by a covalent bound. The conjugate associates the characteristics and biological activities of each component. Uptima provides imiodester high quality crosslinkers with different arm to separate conjugated molecules.

- These cross-linkers are **water soluble** and membrane permeable.
  Solubility is >50mg (DMA, DMS)/ml in water, and 5%(DMS) in methanol
- The **imido ester** group reacts with primary amine groups to form at pH7-10 imido amides bound (amidine) .
- The **reaction** is specific to amines at pH7-9, but above pH9.5, a reaction may occur with epsilon-amines
- Unlike other amine reactive groups (i.e. succinimidyl group), it bears an effective 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 bound 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 arms** of the 3 cross-linkers differ by the length, providing tools for the study of protein to protein interactions, (Pepsinky 1980) and of oligomeres viccinity. 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).

Guidelines for Use

Protocol 1 : Conjugating proteins and peptides

This protocol is a guideline to conjugating 2 proteins, or 2 amine-containing molecules. It is important to check that the molecules to be coupled are pure enough.

1- Prepare and mix proteins or peptides to be conjugated at 1-5 mg/ml in 0.2 M borate 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 proteins solution. Incubate for 1 hour at +4°C under agitation.
The lower protein concentration is, the higher the molar excess. the excess should be optimized for each protein and each application.
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

The protocol is a guideline to immobilize antibodies onto a proteinA beads. The formed bonds are not very stable a high pH values, and can even be reversed. For obtained a stronger immobilization of antibodies, use other coupling strategies (NHS, EDC mediated amidation, hydrazone chemistry).

1- mix antibodies with protein A - beads and allow to bind at room temperature for 30min-1 hr (on roller)
Use 2 mg of antibody per ml wet beads functionnalized by protein A or G.

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.

4- add solid DMP (dimethylpimelimidate) to a final concentration of 20 mM [52 mg for 10 ml]
DMP will corrslink the antibody to proteinA

5- stop reaction by washing the beads twice in 0.2 M ethanolamine pH 8.0

6- incubate on roller for 2 hr at room temperature in 0.2 M ethanolamine pH 8.0

7- 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

rem: on can remove equivalent of 10 µl beads after step 2 (= before sample) and after step 4 (= after sample), to perform controls.

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 Greaves, M.F. (1982). A one-step purification of membrane proteins using a high efficiency immunomatrix. J. Biol. Chem. 257(18), 10766-10769.

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

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

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

Related products :

CelluSep™ dialysis : for desalting

Other cleavable homobifunctional amine reactive crosslinkers: DSP UP18971A and DTPB UP18628A (thiol cleavable), DST UP28068A (oxidizer cleavable), EGS UP28067A (mild alkaline cleavable)…

rev.: N11E-M01E-B07E