EDAC (carbodiimide)
Heterobifunctional cross-linker

Product Description

EDAC is generally utilized as a carboxyl activating agent for amide bonding with primary amines. In addition, it will react with phosphate groups. It has been used in peptide synthesis; crosslinking proteins to nucleic acids; and preparation of immunoconjugates as examples. Typically, it is utilized in the pH range 4.0-6.0.

Catalog number: UP52005A, 5 g  UP52005B, 25 g  UP52005C, 100 g

Name: EDAC, EDC, carbodiimide

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Formula: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride
C₈H₁₇N₃-HCl, CAS: 25952-53-8, M.W.= 191.7

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

General Considerations

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

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 crosslinkers 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 polymères. The choice of reactivities is determinant to the design of the right conjugate.

Carbodiimides react with carboxyls to form an intermediate that can stabilize with reaction with amines, forming a peptidic bond, without spacer lenght.

Uptima offers a high quality EDAC to answer the needs of coupling proteins and peptides for biological and immunoassays like:
- Obtention of immunogens carrier-hapten
- Obtention of labeled affine probes
- Obtention of oligomeric conjugates : conjugates of oriented peptides (through their terminal COOH) for immunization, dimeric or reticulated proteins for structural studies (Ferreira 1994, Wilkens 1998), polymerization, grafting haptens …
- Crosslink for structural studies, with intramolecular, inter sub-unit or between proteins and DNA (Thomas 1978).
- Obtention of biologically active conjugates: specific antibody coupled to drugs for immunotargetting techniques, immunotoxins, …
- Im mobilization of peptide, proteins, sugars.. to various supports: polystyrene plates, beads, gels, biosensors (Burgener 2000, Aebersold 1990)…
- fixation for immunohistodetections (Panula 1988, Tymianski 1997 )
- Stabilization of molecules by reticulation (stabilize allophycocyanin in its allophycocyanin conjugates)

Ask Uptima for other crosslinkers.
Protocole 1: Conjugating a peptide to a protein carrier
This standard protocol can be applied to proteins.

- Prepare the carrier protein at 10mg/ml in MES 0.1M pH5
  On may prefer high capacity coupling carriers (MaxiBind-BSA, MaxiBind-OVA, MaxiBind-blLG) to classic carriers (BSA #UP909382, KLH #UP85502, Ovalbumin UPR5851).
  Desalting may be required to remove unsuitable buffers (Tris) or interfering substances (DTT or other thiols, amines, acetate. Use CelluSep dialysis, or Desalting columns.
- Prepare the peptide or hapten in MES buffer
- Prepare the EDAC at 10mg/ml in distilled water.
  Note: This working solution should be use immediately
- Add 2mg peptide to 2mg carrier
  Note: The carrier / hapten incubation ratio may be optimized depending the desired coupled ratio.
- Add 0.5-1mg of EDAC to the carrier/hapten mixture (0.05-0.4mg for each mg of total protein)
  Note: The EDAC / carrier may be optimized depending on their nature and MW. Usually, 0.5mg of EDAC suits for 1mg BSA + 1mg peptide, and 0.25mg for 1mg KLH + 1mg peptide.
- Incubate for 2-3hours at room temperature
- Desalt (Use CelluSep dialysis, or Desalting columns) and store to any suitable buffer (usually in PBS UP30715)

The conjugate may be frozen until use. For immunizations, it is not necessary to filter to remove eventual precipitates.

Protocole 2: Conjugating a peptide to a protein carrier
This standard protocol can be applied to proteins, and other polystyrene supports bearing carboxyls.

- Wash 1ml (100mg/ml) of carboxyls bearing microspheres (often supplied at 10% solid) in 10ml of activation buffer pH4.5-7.5 (Phosphate, acetate…; the reaction/hydrolysis rate of EDAC increases with lower pH)
- After second wash; resuspend pellet in 10ml of activation buffer ensuring that the microspheres are completely suspended (vortex, sonicate should suffice) at 10mg/ml
  while mixing, add 100mg of EDAC
- Allows to react for 15min at room temperature under continuous mixing
- Wash twice in coupling buffer pH7.2 -8.5 (avoid amine containing buffers like Tris and Glycine), and resuspend in 5ml of same. Ensure that the particles are completely resuspended.
- Dissolve protein (1-10x excess of calculated monolayer *) in 5ml coupling buffer. Combine microsphere suspension and protein mixing
  *Monolayer is for example 18mg of BSA, or 15mg of IgG to saturate 1g of 1µm microspheres
- Wash, resuspend in 10ml of quenching solution, and mix gently for 30 minutes. Wash, and resuspend in storage buffer (i.e. PBS pH7.4 with 0.1% azide and 0.5% BSA)
- Store at +4°C until used, do not freeze.

Note: One-step coupling reactions (EDAC, protein and microsphere combined in one step), are often problematic for coupling larger molecules, but has been used effectively for smaller like steroids and haptens (Nathan, Hage, Quask)

ml

Scientific and Technical Information

- EDAC is soluble up to 100mg/ml in water.
- Following is information related mainly to it’s use as condensing agent.
- Carbodiimide reacts with carboxyls to give an intermediate o-acylisourea. the reaction take place a in an acidic buffer (pH 4.7-5.5), but coupling can actually be accomplished in a buffer system up to pH 7.4.
- The intermediate undergoes hydrolysis in aqueous solutions, thus a stabilization is usually necessary for further coupling to amines. A classic way to do it consists to add N-hydroxysuccinimide (UP04594) (Grabarek 1990, Staros 1986). Hydrolysis by-product is a N-substituted urea, the carboxyl being regenerated to it’s original form.
• The intermediate reacts with amines, forming a peptidic bonded conjugate. It reacts also with hydrazide, allowing to use hydrazide activated ligands (Biotin Hydrazide UP36466, or hydrazide activated supports) to label or graft ligands through their carboxyls residues.

• A side reaction may take place, notably with carboxyls in hydrophobic environment, giving a N-acyl-urea.

• To reduce intra- and interprotein coupling to lysine residues, which is a common side reaction, carbodiimide-mediated coupling should be performed in a concentrated protein solution at a low pH, using a large excess of the nucleophile.

• The EDAC-mediated coupling has the particularity to form no spacer between the 2 molecules. The formed peptidic bond is homologous to natural protein and peptides, what is taken to good account for peptide synthesis, and while other crosslinkers generated bonds are often recognized as foreign molecules.

• Reaction time are reduced in MES(#14035B) buffer during the EDAC/NHS activation step. A higher pH (up 7-8 will increase also the kinetic, but also competitive hydrolysis).

• The ratio of coupling may be estimated by specific assays (if specific probes are available for at least one molecule), or physical measurements (i.e. if the peptide is rich in tyrosine residues, A280nm of the conjugate may be compared to A280nm of the carrier alone).

• EDAC has been shown to be impermeable to membranes of live cells, which permits its use to distinguish between cytoplasmic and lumenal sites of reaction. EDAC may be useful for conjugating fluorescent aliphatic amines to cell-surface proteins.

• Easy removal of excess reagent and corresponding urea after coupling may be achieved by washing with dilute acid or water.

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**


*PubMed* Lin CM, Mihal KA, Krueger RJ. Biochim Biophys Acta 1038, 382-385 (1990) PN12792. [with cysteamine, the DTT reduction of S-S] "Introduction of sulphydryl groups into proteins at carboxyl sites."


Sheehan J.C. and Ledis, Total synthesis of a monocyclic peptide lactone antibiotic, etamycin J. Am. Chem. Soc. 95, 875-879 (1973)