FT-UP52005

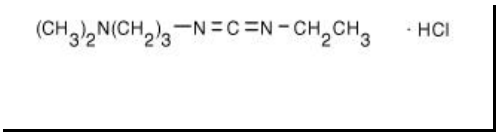
EDAC (carbodiimide)

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**
Formula: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride
$C_8H_{17}N_3$-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 bound. 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 dimeres and polymeres. 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, graffting haptens …
- Crosslink for stuctural 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, …
- Immobilization 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.

Use

Protocole 1: Conjugating a peptide to a protein carrier
This standard protocole 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-bLG) to classic carriers (BSA #UP909382, KLH #UP88502, 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 filtrate to remove eventual precipitates.

Protocole 2: Conjugating a peptide to a protein carrier
This standard protocole 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 microsphers 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 w<ith 0.1% azide and 0.5% BSA)
- Store at +4°C until usded, 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 eefectively 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 aquous 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-substitued 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(Lewis 1994).
- 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 (Renthal 1987). 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 contat Uptima, or your local distributor.
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Literature

PubMed Aebersold R, Pipes GD, Wettenhall RE, Nika H, Hood LE. Anal Biochem 187, 56-65 (1990) PN10490.· "Covalent attachment of peptides for high sensitivity solid-phase sequence analysis."
PubMed Angelotti TP, Clarke MF, Longino MA, Emerson SG. Bioconjug Chem 2, 466-474 (1991) PN12951.·"Biotinylated granulocyte/macrophage colony-stimulating factor analogues: effect of linkage chemistry on activity and binding."
PubMed Bauminger S, Wilchek M. Methods Enzymol 70, 151-159 (1980) PN12837.· "The use of carbodiimides in the preparation of immunizing conjugates."
PubMed Beckers G, Berzborn RJ, Strotmann H. Biochim Biophys Acta 1101, 97-104 (1992) PN31842.· "Zero-length crosslinking between subunits delta and I of the H(+)-translocating ATPase of chloroplasts."
PubMed Blotnick E, Muhlrad A. Biochemistry 33, 6867-6876 (1994) PN17901.· "Effect of nucleotides and actin on the intramolecular cross-linking of myosin subfragment-1."
PubMed Burgener M, Sanger M, Candrian U. Bioconjug Chem 11, 749-754 (2000) PN39817.· "Synthesis of a stable and specific surface plasmon resonance biosensor surface employing covalently immobilized peptide nucleic acids."
PubMed Ferreira JP, Sasisekharan R, Louie O, Langer R. Eur J Biochem 223, 611-616 (1994) PN33539.· "Carbodiimide modification enhances activity of pig pancreatic phospholipase A2."
PubMed Gilles MA, Hudson AQ, Borders CL Jr. Anal Biochem 184, 244-248 (1990) PN45289.· " Stability of water-soluble carbodiimides in aqueous solution."
PubMed Girardot JM, Girardot MN. J Heart Valve Dis 5, 518-525 (1996) PN31575.· "Amide cross-linking: an alternative to glutaraldehyde fixation."
PubMed Grabarek Z, Gergely J. Anal Biochem 185, 131-135 (1990) PN45288. "Zero-length crosslinking procedure with the use of active esters."
PubMed Kobayashi M, Chiba Y. Anal Biochem 219, 189-194 (1994) PN17669.· "Water-soluble carbodiimide for the fluorescent measurement of the carboxyl group produced by enzyme reactions."
PubMed Kobayashi M, Ichishima E. Anal Biochem 189, 122-125 (1990) PN11729.· "Use of water-soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide for the fluorescent determination of uronic acids and carboxylic acids."
PubMed Kuroki R, Yamada H, Imoto T. J Biochem (Tokyo) 99, 1493-1499 (1986) PN12804.· " Specific carbodiimide-binding mechanism for the selective modification of the aspartic acid-101 residue of lysozyme in the carbodiimide-amine reaction."
PubMed Lewis MR, Raubitschek A, Shively JE. Bioconjug Chem 5, 565-576 (1994) PN33542.· "A facile, water-soluble method for modification of proteins with DOTA. Use of elevated temperature and optimized pH to achieve high specific activity and high chelate stability in radiolabeled immunoconjugates."
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 sulfhydryl groups into proteins at carboxyl sites."

PubMed Millan KM, Mikkelsen SR. Anal Chem 65, 2317-2323 (1993) PN33536.· "Sequence-selective biosensor for DNA based on electroactive hybridization indicators."
PubMed Ono S, Benian GM. J Biol Chem 273, 3778-3783 (1998) PN29317.· "Two Caenorhabditis elegans actin depolymerizing factor/cofilin proteins, encoded by the unc-60 gene, differentially regulate actin filament dynamics."
PubMed Panula P, Happola O, Airaksinen MS, Auvinen S, Virkamaki A. J Histochem Cytochem 36, 259-269 (1988) PN45850.· "Carbodiimide as a tissue fixative in histamine immunohistochemistry and its application in developmental neurobiology."
PubMed Renthal R, Cothran M, Dawson N, Harris GJ. Biochim Biophys Acta 897, 384-394 (1987) PN5855.· "Fluorescent labeling of bacteriorhodopsin: implications for helix connections."
PubMed Staros JV, Wright RW, Swingle DM. Anal Biochem 156, 220-222 (1986) PN12691.· "Enhancement by N-hydroxysulfosuccinimide of water-soluble carbodiimide-mediated coupling reactions."
PubMed Thomas JO, Sternberg N, Weisberg R. J Mol Biol 123, 149-161 (1978) PN19955.· "Altered arrangement of the DNA in injection-defective lambda bacteriophage."
PubMed Tymianski M, Bernstein GM, Abdel-Hamid KM, Sattler R, Velumian A, Carlen PL, Razavi H, Jones OT. Cell Calcium 21, 175-183 (1997) PN28187.· "A novel use for a carbodiimide compound for the fixation of fluorescent and non-fluorescent calcium indicators in situ following physiological experiments."
PubMed Wilkens S, Capaldi RA. J Biol Chem 273, 26645-26651 (1998) PN31759.· " Solution structure of the epsilon subunit of the F1-ATPase from Escherichia coli and interactions of this subunit with beta subunits in the complex."
Dhaon M.K., Synthesis of esters J. Org. Chem. 47, 1962 (1982)
Drabick J.J. et al., Covalent polymyxin B conjugate with human immunoglobulin G as an antiendotoxin reagent Antimicrob. Agents Chemother. 42, 583-588 (1998)
Sheehan J.C. and Ledis, Total synthesis of a monocyclic peptide lactone antibiotic, etamycin J. Am. Chem. Soc. 95, 875-879 (1973)
Thomas J.O. et al., Altered arrangement of the DNA in injection-defective lambda bacteriophage. J. Mol. Biol. 123, 149 (1978)
Lundblad R.L. et al, Modification of carboxyl groups in proteins, review Chemical Reagents for Protein Modification 2, 105 (1984)
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