FP-52005D

EDAC (carbodiimide) Heterobifunctionnal cross-linker

Description

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

Catalog number: FP52005D, 1 g

Name: **EDAC, EDC, EDAP, EDCI, carbodiimide**

Formula : 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride

C_8H17N_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 length.

FluoProbes offers a high quality EDAC to answer the needs of coupling proteins and peptides for biological and immunoassays like:

- Preparation of immunogens carrier-hapten
- Preparation of labeled affine probes
- Preparation 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 stuctural studies, with intra-molecular, inter sub-unit or between proteins and DNA (Thomas 1978).
- Preparation 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 FluoProbes for other crosslinkers.

Use

Protocol 1: One step Conjugation of a peptide to a protein

This standard protocol can be applied to most proteins and peptides, i.e. for creating immunization carrier-hapten conjugates.

- 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.

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)

Protocol 2: Two steps Conjugation of a peptide to a protein (Grabarek 1990)

Two steps method create a better definite and 'oriented' conjugate, because it avoids to expose the second protein to EDCC, thus to modify it's carboxyls. However the first protein should bear a reducing step (to block the activation).

- Prepare protein at 1mg/ml in suitable buffer (i.e. 0.1 M MES, 0.5 M NaCl, pH 6.0) and add 2mM EDC + 0.6mM NHS UP39044 (or sNHS UP52117).
- Incubate for 15minutes at room temperature, ad stop by adding 20mM DTT (UP28425).

Note: These activation conditions may be optimized by tested different ratios of EDC and HNS, and using a pH5 buffer.

- Desalt the activated protein with desalting columns (UP84874) or other mean (optional)
- Add the second protein at 1:1 molar ratio.
- Incubate for 2hours at room temperature.

Note :The reaction rate may be increased rising pH up 8.5 but hydrolysis will also be increased.

- Quench excess NHS by adding 10mM of hydroxylamine (1hour incubation)
- Desalt the conjugate with desalting columns (UP84874) or other mean.

Protocol 3: Conjugating a peptide to a polystyrene support

This standard protocol can be applied to polystyrene supports bearing carboxyls (microplates, beads…).

- 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; re-suspend pellet at 10mg/ml in 10ml of activation buffer ensuring that the microspheres are completely suspended (vortex, sonicate should suffice) while mixing
- Add 100mg of EDAC, and 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 re-suspend in 5ml of same. Ensure that the particles are completely re-suspended.

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- 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, re-suspend in 10ml of quenching solution, and mix gently for 30 minutes. Wash, and re-suspend in storage buffer (i.e. PBS pH7.4 with 0.1% azide and 0.5% BSA).

Protocole 4: Carbodiimide condensation of phosphopeptides
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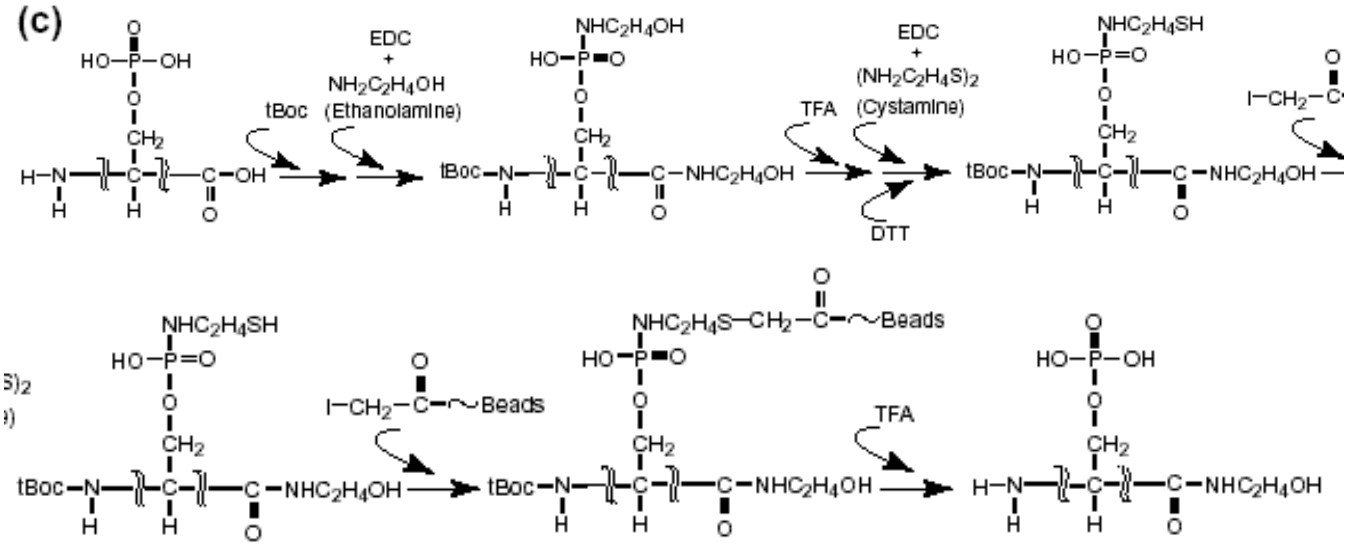

This reaction scheme has been useful for phosphopeptides isolation and analysis (reduction/alkylation of cysteine; trypsin digestion/Amide protection by t-boc/carbodiimide condensation/phosphate regeneration. Cysteamine derivatization/SH immobilization/elution)

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 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 bonds generated by other crosslinkers 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

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Other information

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