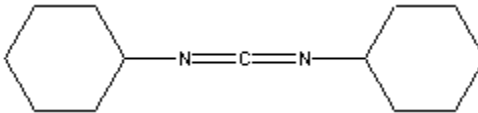
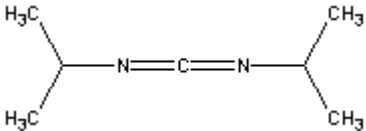


Carbodiimides (EDAC, DCC, DIC)

Heterobifunctional cross-linkers

Products Description

Catalog number:	UP52005A, 5 g UP52005B, 25 g UP52005C, 100 g, UP52005E, 1 Kg	
Name:	EDAC, EDC, EDAP, EDCI, carbodiimide	
Formula :	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride; 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride C ₈ H ₁₇ N ₃ -HCl; CAS: 25952-53-8; MW= 191.7	$(\text{CH}_3)_2\text{N}(\text{CH}_2)_3-\text{N}=\text{C}=\text{N}-\text{CH}_2\text{CH}_3 \cdot \text{HCl}$
Catalog number:	HG9911, 100 g HG9912, 500 g	
Name:	DCC	
Formula :	N,N'-Dicyclohexylcarbodiimide C ₁₃ H ₂₂ N ₂ ; CAS: [538-75-0]; MW= 206.3	
Catalog number:	HG9820, 5 g HG9821, 25 g	
Name:	DIC	
Formula :	N,N'-Diisopropylcarbodiimide; 1,3-Diisopropylcarbodiimide C ₇ H ₁₄ N ₂ ; CAS: [693-13-0]; MW= 126.2	
Storage :	+4°C (shipped at RT, long term storage at -20°C) (κ), protect from moisture and light.	

General Considerations

Cross-linkers are chemical reagents used to conjugate molecules together by a covalent bound.

Heterobifunctional crosslinkers have 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 crosslinkers react with carboxyls to form an intermediate that can stabilize upon reaction with amines, forming a peptidic bond, without spacer. They are typically used in peptide synthesis; cross-linking proteins to nucleic acids; and preparation of immunoconjugates as examples. They also give other reactions and have other uses.

The most popular carbodiimide is **EDAC** because of its water-solubility, making it useful in most applications. **DCC** and **DIC** are water insoluble carbodiimides, and still useful in some synthesis applications, the latter being easier to handle and less allergenic.

Applications include:

- 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](#), [Wilkins 1998](#)), polymerization, grafting haptens ...
- Crosslink for structural 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)

Directions for Use

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

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
One may use high capacity coupling carriers (MaxiBind Proteins) or classic carriers (BSA #UP909382, KLH #UP88502, Ovalb #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 (GS296).
- 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 (i.e. using CelluSep dialysis) 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, Ouask](#)).

Protocol 2: Two steps Conjugation of a peptide to a protein using EDAC ([Grabarek 1990](#))

Two steps method create a better definite and 'oriented' conjugate, because it avoids to expose the second protein to EDC, 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)
 - Add 2mM EDC + 0.6mM NHS ([UP04594A](#)) (or sNHS UP54422).
Incubate for 15minutes at room temperature, and stop by adding 20mM DTT (UP28425).
- Note: For 1ml of protein at 1mg/ml, this makes ≈0.4mg EDC and ≈0.6mg of NHS.
These activation conditions may be optimized by testing different ratios of EDC and NHS (i.e. 1-2 concentrations above and below), and using a pH5 buffer.
- Desalt the activated protein with desalting columns (UP84874) or other mean (CelluSep dialysis)
 - 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 using EDAC

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

- Wash 1ml (100mg/ml) of carboxyls bearing microspheres (often supplied as 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
- Note: Ensure 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
- Note: avoid amine containing buffers like Tris and Glycine), and re-suspend in 5ml of same. Ensure that the particles are completely re-suspended.
- 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).

Protocol 4: Carbodiimide condensation of phosphopeptides

Organic phosphate groups (i.e. 5'-end phosphate of nucleic acids) can be coupled using EDC.

Reaction schemes include:

- Activation of the 5'-end phosphate group with carbodiimide stabilized by EthyleneDiamine
1-Methylimidazole can also be used to get a phosphorylimidazolide group that will be reactive in presence of excess EthyleneDiamine forming Phosphoramidate bond. Depending on the amine-containing molecules used, the crosslinking strategy can be adapted in a number of ways to directly or indirectly modify, label or conjugate an oligonucleotide:

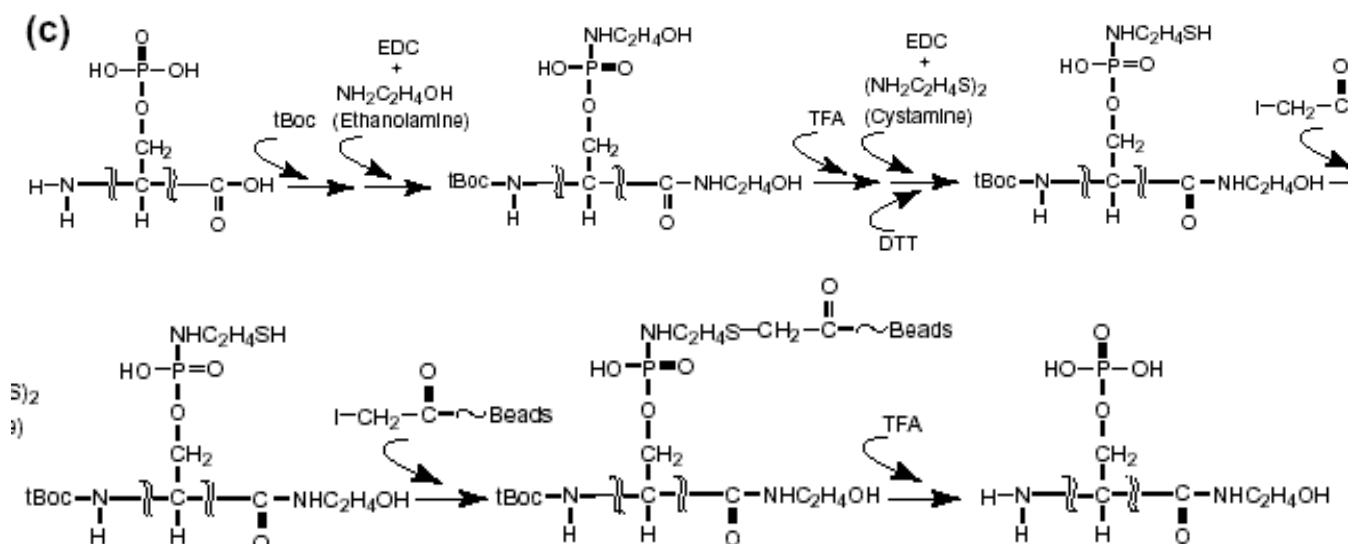
Use cystamine instead of ethylenediamine in the default reaction, and then reduce the disulfide bond with a reductor such as DTT, to create sulfhydryl groups (used further to create oligo-enzyme conjugates)

Use Hydrazide derivatives (i.e. of Biotin #UP68631 or of FluoProbes) instead of ethylenediamine in the default reaction.

Use ABH UP87750 instead of ethylenediamine in the default reaction to conjugate regardless of the chemical type, though a photoreactive group.

Use EMCH 90038 or analogs instead of ethylenediamine in the default reaction to prepare conjugates with sulfhydryl-containing proteins and other molecules. Use PDPH (UP99648 for a reversible conjugation (cleavable spacer).

- 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). Reference: TRENDS in Biotechnology Vol 20, June 2002



Use similar strategies to prepare affinity purification supports.

Scientific and technical Information

Carbodiimides primary use is solid-phase peptide synthesis and peptide coupling at the N-terminus. They enhance the electrophilicity of the negatively charged oxygen of carboxylate group, thus activate it a better leaving group. The activated intermediate makes more efficient the nucleophilic attack by the terminal amino group on the growing peptide.

DCC is a waxy solid.

It is a dehydrating agent for the preparation of amides, ketones, nitriles. DCC can also dehydrate Alcohols.

DCC catalyse the reaction of an acid with hydrogen peroxide to form a peroxide linkage.

DCC in dimethyl sulfoxide (DMSO) realize the Pfitzner-Moffatt oxidation of alcohols to aldehydes and ketones, in a sufficiently mild way to avoid over-oxidation of aldehydes to carboxylic acids (unlike metal-mediated

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oxidations). Generally, three equivalents of DCC and 0.5 equivalent of proton source in DMSO are allowed to react overnight at room temperature. The reaction is quenched with acid.

DCC can be used to invert stereoisomers of a secondary alcohol after saponification (it is added with formic acid and a strong base such as sodium methoxide).

A range of alcohols, including even some tertiary alcohols, can be esterified using a carboxylic acid in the presence of DCC and a catalytic amount of DMAP ¹.

DIC is identical to DCC in many ways, but is easier to handle (a liquid), and can be removed by extraction (not by filtration). It is also less allergenic. DiC is a liquid and the urea byproduct is organic soluble so it is useful when your end product is water soluble.

EDAC (EDC) is an even easier to handle solid that is water soluble (>200g/l). It is used in a wide range of solvents under mild conditions eg water, DCM, THF and DMF. The advantage over DCC is that the urea by product is water soluble and easily removed by extraction. EDAC has become the most popular carbodiimide used as condensing agent between NH₂ and COOH groups.

Carbodiimide reacts with carboxyls to give an intermediate o-acylisourea. The reaction takes place in an acidic buffer (pH 4.7-5.5), but coupling can actually be accomplished in a buffer system up to pH 7.4. Typically, it is utilized in the pH range 4.0-6.0.

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 its original form.

The intermediate reacts with amines, forming a peptidic bonded conjugate.

A side reaction may take place, notably with carboxyls in hydrophobic environment, giving a N-acyl-urea. To reduce intra- and inter-protein 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. Slightly acid conditions are used, because higher pH (up 7-8) increase 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 luminal 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.

EDC can react with phosphate groups, and alcohols.

Depending on the amine-containing molecules used, the crosslinking strategy can be adapted in a number of ways to directly or indirectly modify, label or conjugate a protein or an oligonucleotide.

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Literature - DIC

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Regulatory Information - DCC

UN:2928 Highly toxic.

Xn Corrosive material

- R22- Harmful if swallowed.
- R24- Toxic in contact with skin.
- R41- Risk of serious damage to eyes.
- R43- May cause sensitization by skin contact.
- S1/2- Keep locked up and out of the reach of children.
- S24- Avoid contact with skin.

- S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- S37/39- Wear suitable gloves and eye/face protection.
- S45- In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

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

For any information, please contact Uptima, or your local distributor.

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