



526PR-02

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A Geno Technology, Inc. (USA) brand name

EDC

1-ethyl-3-(3-dimethylamino) propyl carbodiimide,
hydrochloride

(Cat. # BC25-50, BC25-1, BC25-5, BC25-25)



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INTRODUCTION

EDC is a heterobifunctional, water-soluble, zero-length carbodiimide crosslinker that is used to couple carboxyl groups to primary amines. EDC activates carboxyl groups first and forms amine reactive *O*-acylisourea intermediate that spontaneously reacts with primary amines to form an amide bond and isourea by-product.

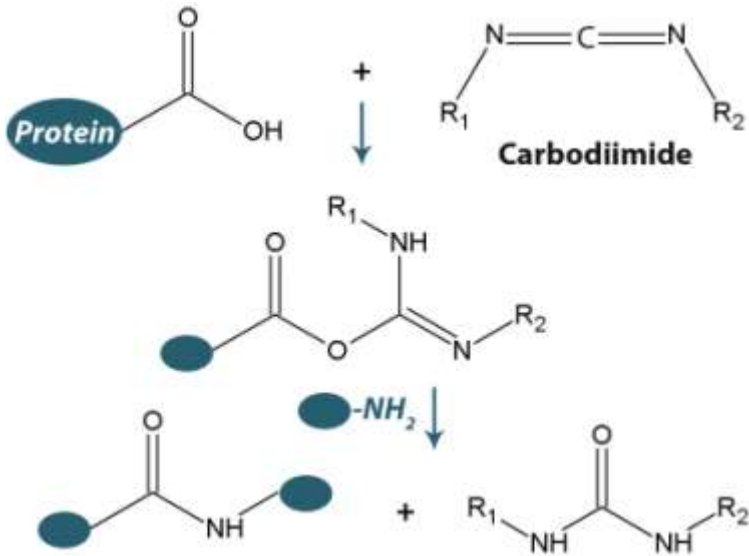


Figure 1: EDC Coupling Scheme

The unstable nature of the intermediate in aqueous solutions makes 2-step coupling, however in conjunction with *N*-hydroxysuccinimide, a 2-step coupling is possible.

EDC is ideal for peptide immobilization and hapten-carrier protein conjugation.

ITEM(S) SUPPLIED

Cat. #	Description	Size
BC25-50	EDC	50mg
BC25-1	EDC	1g
BC25-5	EDC	5g
BC25-25	EDC	25g

STORAGE CONDITIONS

EDC is shipped at ambient temperature. Upon arrival, store at -20°C in the presence of a desiccant.

ADDITIONAL MATERIAL(S) REQUIRED

- Carrier Protein (2mg)
We recommend our OneQuant™ BSA, HyperCarrier™ and keyhole limpet hemocyanin (KLH) Carrier Proteins (Cat. # 786-090, 786-092, 786-091 respectively).
- Conjugation Buffer
0.1M MES, pH4.5-5 or G-Biosciences Optimizer Buffer-IV (Cat. # BKC-07)
- Hapten or peptide (1-2mg)
- Desalting column
We recommend SpinOUT™ GT-600, 3ml (Cat. # 786-171)

PROTOCOL

1. Equilibrate the EDC to room temperature before opening.
NOTE: EDC is highly hygroscopic; failure to allow to equilibrate may lead to poor cross linking.
2. Add 2mg carrier protein to 500µl Conjugation Buffer.
3. Dissolve up to 2mg hapten or peptide in 500µl Conjugation Buffer and add to the protein solution.
4. For HyperCarrier™, BSA or ovalbumin, dissolve 10mg EDC in 1ml deionized water and immediately add 100µl EDC solution to the protein:hapten solution.
For KLH, dissolve 10mg EDC in 1ml deionized water and immediately add 50µl EDC solution to the protein:hapten solution. If precipitation occurs, reduce the amount of EDC solution added further.
5. Incubate at room temperature for 2 hours.
6. Purify the coupled protein and hapten using a desalting column. We recommend our SpinOUT™ desalting columns.

APPENDIX 1: 2-STEP COUPLING WITH EDC AND NHS

Introduction

The following protocol allows for the sequential coupling of two proteins without affecting the second protein's carboxyls by quenching the first reaction with a thiol containing compound.

Additional Material(S) Required

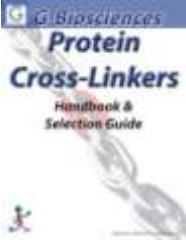
- Conjugation Buffer 1
0.1M MES, pH4.5-5 or G-Biosciences Optimizer Buffer-IV (Cat. # BKC-07)
- Conjugation Buffer 2: *1X PBS*
- Protein #1 (1mg/ml), prepared in Conjugation Buffer 1
- Protein #2 (1mg/ml), prepared in Conjugation Buffer 2
- NHS or Sulfo-NHS (Cat. # BC97)
- Desalting column
We recommend SpinOUT™ GT-600, 3ml (Cat. # 786-171)
- 2-Mercaptoethanol
- Hydroxylamine.HCl

Protocol

1. Equilibrate the EDC and NHS to room temperature before opening.
NOTE: *These are highly hygroscopic; failure to allow to equilibrate may lead to poor cross linking.*
2. Prepare 1ml of a 1mg/ml solution of Protein #1 in Conjugation Buffer 1.
3. Add 0.4mg EDC and 0.6mg NHS or 1.1.mg sulfo-NHS and react for 15 minutes at room temperature.
4. Add 1.2µl 2-mercaptoethanol to quench the EDC.
NOTE: *At this stage the protein can be separated from excess 2-mercaptoethanol with a desalting column.*
5. Add an equimolar amount of Protein #2 compared to Protein#1 and allow to react at room temperature for 2 hours.
6. Quench the reaction with the addition of hydroxylamine to a 10mM final concentration.
7. Purify the coupled proteins using a desalting column. We recommend our SpinOUT™ desalting columns.

RELATED PRODUCTS

Download our Protein Cross Linker Handbook.



<http://info.gbiosciences.com/complete-protein-cross-linkers-handbook>

For other related products, visit our website at www.GBiosciences.com or contact us.

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