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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)

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 imtermediate that spontaneously reacts with primary amines to form an amide bond and isourea by-product.

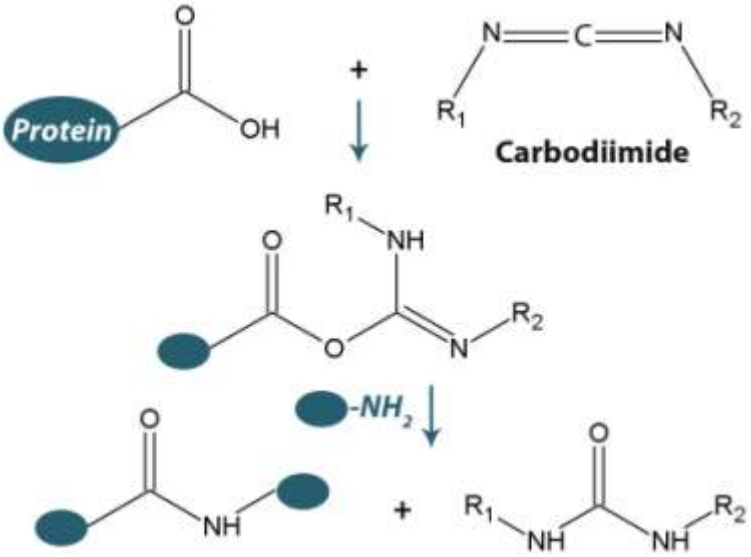


Figure 1: EDC Coupling Scheme

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

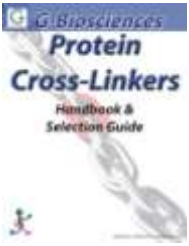
- Conjugation Buffer 1
  0.1M MES, pH4.5-5 or G-Biosciences Optimizer Buffer-IV (Cat. # BKC-07)
- Conjugation Buffer 2: *1X PBS*
- Protein #1 (1mg/ml), prepared in Conjugation Buffer 1
- Protein #2 (1mg/ml), prepared in Conjugation Buffer 2
- NHS of Sulfo-NHS (Cat. # BC97)
- Desalting column
  We recommend 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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