INSTRUCTIONS

EDC

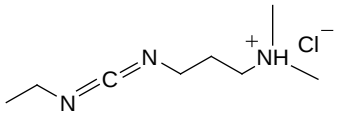
MAN0017125
Rev. A.0
Pub. Part No. 2160475.7

22980 22981 77149

Number	Description
22980	**EDC** (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride), 5g
22981	**EDC,** 25g
77149	**EDC,** 10mg

Molecular Weight: 191.7

CAS # 25952-53-8



Storage: Upon receipt store desiccated at -20°C. Products are shipped with an ice pack.

For Research Use Only. Not for use in diagnostic procedures.

Introduction

Thermo Scientific™ EDC is a carboxyl and amine-reactive zero-length crosslinker. EDC reacts with a carboxyl group first and forms an amine-reactive *O*-acylisourea intermediate that quickly reacts with an amino group to form an amide bond and release of an isourea by-product (see the Additional Information Section). The intermediate is unstable in aqueous solutions, and therefore, two-step conjugation procedures rely on *N*-hydroxysuccinimide for stabilization.[1,2] Failure to react with an amine will result in hydrolysis of the intermediate, regeneration of the carboxyl and release of an *N*-substituted urea. A side reaction is the formation of an *N*-acylurea, which is usually restricted to carboxyls located in hydrophobic regions of proteins.[1,3]

Procedure for Using EDC for Coupling Haptens to a Carrier Protein

Materials Required

- Carrier protein: 2mg bovine serum albumin (BSA), ovalbumin (OVA) or keyhole limpet hemocyanin (KLH)
- Conjugation Buffer: 0.1M MES (2-[*N*-morpholino]ethane sulfonic acid), pH 4.5-5 (Product No. 28390)
- EDC: 10mg
- Hapten: 1-2mg
- Thermo Scientific™ Zeba™ Spin Desalting Column (Product No. 89891) or other gel filtration column with a 5-6K molecular-weight cutoff

Procedure

1. Equilibrate EDC to room temperature.
2. Add 2mg of lyophilized BSA, OVA or KLH to 200µL Conjugation Buffer. If using Thermo Scientific™ Imject™ Carrier Proteins, reconstitute using ultrapure water.
3. Dissolve up to 2mg of the peptide or hapten in 500µL of Conjugation Buffer and add it to the 200µL carrier protein solution.
4. For BSA or OVA conjugation, dissolve 10mg of EDC in 1mL of ultrapure water and immediately add 100µL of this solution to the carrier-peptide solution. For KLH conjugation, dissolve 10mg of EDC in 1mL of ultrapure water and immediately add 50µL of this solution to the carrier-peptide solution. Further reduce the amount of EDC if precipitation occurs.
5. React for 2 hours at room temperature.
6. Purify the conjugate using a desalting column. If storing the immunogen for more than a few days, sterile filter the conjugate and store in a sterile container at 4°C or -20°C.

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Procedure for Two-step Coupling of Proteins Using EDC and NHS or Sulfo-NHS

The following protocol, adapted from a procedure described by Grabarek and Gergely, allows sequential coupling of two proteins without affecting the second protein's carboxyls by exposing them to EDC. This procedure requires quenching the first reaction with a thiol-containing compound.

The activation reaction with EDC and Sulfo-NHS is most efficient at pH 4.5-7.2; however, the reaction of NHS-activated or Sulfo-NHS-activated molecules with primary amines is most efficient at pH 7-8. For best results, perform the first reaction in MES buffer (or other non-amine, non-carboxylate buffer) at pH 5-6, then raise the pH to 7.2-7.5 with phosphate buffer (or other non-amine buffer) immediately before reaction to the amine-containing molecule. For quenching the first reaction, use 2-mercaptoethanol, or the excess reagent can be simply removed (as well as the reaction pH adjusted) by buffer-exchange with a desalting column.

Materials Required

- Activation Buffer: 0.1M MES, 0.5M NaCl, pH 6.0
- Coupling Buffer: Phosphate-buffered saline (PBS), 100mM sodium phosphate, 150mM NaCl; pH 7.2 (Product No. 28372)
- Protein # 1: Prepared in Activation Buffer at 1mg/mL
- Protein # 2: Prepared in Coupling Buffer
- NHS or Sulfo-NHS (Product No. 24500 and 24510, respectively)
- 2-Mercaptoethanol (Product No. 35600)
- (Optional) Zeba Spin Desalting Column (Product No. 89891) or other gel filtration column
- Hydroxylamine•HCl (Product No. 26103)

Procedure

1. Equilibrate EDC and NHS to room temperature before opening bottles.
2. Add 0.4mg EDC (~2mM) and 0.6mg of NHS or 1.1mg of sulfo-NHS (~5mM) to 1mL of protein #1 solution and react for 15 minutes at room temperature.
3. Add 1.4µL of 2-mercaptoethanol (final concentration of 20mM) to quench the EDC.
4. Optional: Separate the protein from excess reducing agent and inactivated crosslinker using a desalting column that has been equilibrated with Coupling Buffer (PBS).
5. Add protein #2 to the activated protein at an equal molar ratio with protein #1. Allow the proteins to react for 2 hours at room temperature.
6. To quench the reaction, add hydroxylamine to a final concentration of 10mM. This method hydrolyzes nonreacted NHS present on protein #1 and results in hydroxamate. Other quenching methods involve adding 20-50mM Tris, lysine, glycine or ethanolamine; however, these primary amine-containing compounds modify carboxyls on protein #1.
7. Remove excess quenching reagent using a desalting column.

Additional Information

EDC reacts with a carboxyl group first and forms an amine-reactive *O*-acylisourea intermediate that quickly reacts with an amino group to form an amide bond and release of an isourea by-product (Figure 1).

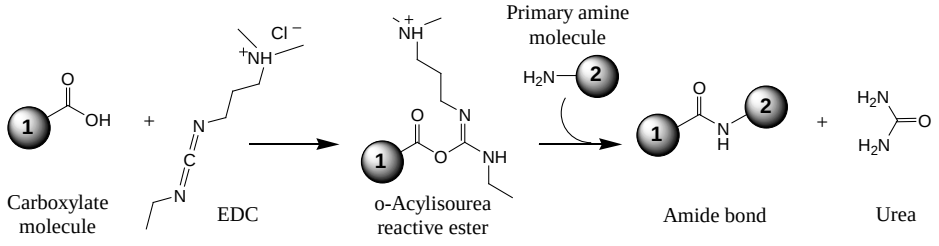

Figure 1. One-step EDC reaction with carboxyl and amine-containing molecules.

Information from Our Website

- Tech Tip #15: Biotinylate carboxyl groups with EDC and Biotin Hydrazide
- Tech Tip #5: Attach an antibody onto glass, silica or quartz surface
- Tech Tip #18: Block amino groups to prevent polymer formation in peptide-carrier protein conjugations
- Tech Tip #30: Modify and label oligonucleotide 5´ phosphate groups
- Tech Tip #3: Determine reactivity of NHS-ester biotinylation and crosslinking reagents
- Tech Tip #46: Preferentially biotinylate N-terminal alpha-amino groups in peptides

Related Thermo Scientific Products

24500	**NHS** (N-hydroxysuccinimide), 25g
24510	**Sulfo-NHS** (N-hydroxysulfosuccinimide), 500mg
24525	**Sulfo-NHS,** 5g
24520	**Sulfo-NHS, No-Weigh™ Format**, 8 × 2mg microtubes
26166	**Bioconjugate Techniques (Book)**
89889	**Zeba Spin Desalting Columns, 2mL,** 5/pkg
89891	**Zeba Spin Desalting Columns, 5mL,** 5/pkg
89893	**Zeba Spin Desalting Columns, 10mL,** 5/pkg
22360	**SMCC** (succinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate), 50mg
22322	**Sulfo-SMCC** (sulfosuccinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate), 50mg
22622	**Sulfo-SMCC, No-Weigh Format**, 8 × 2mg microtubes
21555	**DSS** (disuccinimidyl suberate), 1g
21655	**DSS,** 50mg
21658	**DSS, No-Weigh Format,** 8 × 2mg microtubes
21580	**BS³ (bis[sulfosuccinimidyl] suberate),** 50mg
21585	**BS³, No-Weigh Format,** 8 × 2mg microtubes

General References

Grabarek, Z. and Gergely, J. (1990). Zero-length crosslinking procedure with the use of active esters. *Anal Biochem* **185:**131-5.

Staros, J.V., *et al*. (1986). Enhancement by *N*-hydroxysulfosuccinimide of water-soluble carbodiimide-mediated coupling reactions. *Anal Biochem* **156:**220-2.

Timkovich, R. (1977). Detection of the stable addition of carbodiimide to proteins. *Anal Biochem* **79:**135-43.

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