INSTRUCTIONS

EDC

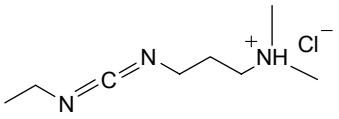
22980 22981 77149

0475.3

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

Molecular Weight: 191.7

CAS # 25952-53-8



Storage: Upon receipt store Product No. 22980 and 22981 desiccated at -20°C. Store Product No. 77149 at 4°C. EDC is shipped at ambient temperature.

Introduction

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 require *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: 2 mg bovine serum albumin (BSA), ovalbumin (OVA) or keyhole limpet hemocyanin (KLH)
- Conjugation Buffer: 0.1 M MES (2-[*N*-morpholino]ethane sulfonic acid), pH 4.5-5 (Product No. 28390)
- EDC: 10 mg
- Hapten: 1-2 mg
- Zeba™ Desalt Spin 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 2 mg of lyophilized BSA, OVA or KLH to 200 µl Conjugation Buffer. If using Pierce Imject® Carrier Proteins, reconstitute using ultrapure water.
3. Dissolve up to 2 mg of the peptide or hapten in 500 µl of Conjugation Buffer and add it to the 200 µl carrier protein solution.
4. For BSA or OVA conjugation, dissolve 10 mg of EDC in 1 ml of ultrapure water and immediately add 100 µl of this solution to the carrier-peptide solution. For KLH conjugation, dissolve 10 mg of EDC in 1 ml of ultrapure water and immediately add 50 µl of this solution to the carrier-peptide solution.
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.

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 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: 0.1 M MES, 0.5 M NaCl, pH 6.0
- Coupling Buffer: Phosphate-buffered saline (PBS), 100 mM sodium phosphate, 150 mM NaCl; pH 7.2 (Product No. 28372)
- Protein # 1: Prepared in Activation Buffer at 1 mg/ml
- Protein # 2
- NHS or Sulfo-NHS (Product No. 24500 and 24510, respectively)
- 2-Mercaptoethanol (Product No. 35600)
- (Optional) Zeba™ Desalt Spin 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 0.4 mg EDC (~2 mM) and 0.6 mg of NHS or 1.1 mg of sulfo-NHS (~5 mM) to 1 ml of protein #1 solution and react for 15 minutes at room temperature.
3. Add 1.4 µl of 2-mercaptoethanol (final concentration of 20 mM) to quench the EDC.
4. Optional: Separate the protein from excess reducing agent and inactivated crosslinker using a desalting column. Equilibrate the column with Coupling Buffer (PBS).
5. Add protein #2 to the activated protein prepared in Coupling Buffer 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 10 mM. This method hydrolyzes nonreacted NHS present on protein #1 and results in hydroxamate. Other quenching methods involve adding 20-50 mM 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).

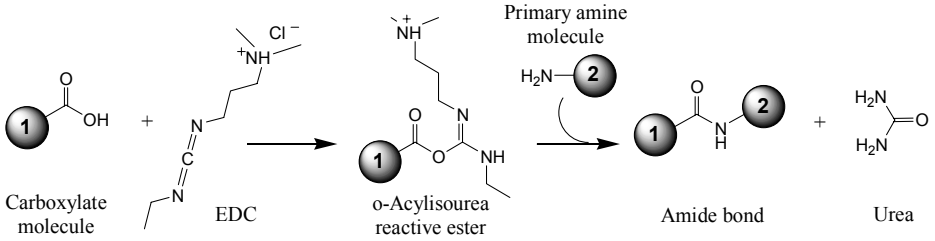


Figure 1. One-step EDC reaction with carboxyl and amine-containing molecules.

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 Products

20002	**Bioconjugate Techniques,** 785 pages, softcover
89889	**Zeba Desalt Spin Columns, 2 ml,** 5 each, for 200-700 µl samples
89890	**Zeba Desalt Spin Columns, 2 ml,** 25 each, for 200-700 µl samples
89891	**Zeba Desalt Spin Columns, 5 ml,** 5 each, for 500-2,000 µl samples
89892	**Zeba Desalt Spin Columns, 5 ml,** 25 each, for 500-2,000 µl samples
89893	**Zeba Desalt Spin Columns, 10 ml,** 5 each, for 700-4,000 µl samples
89894	**Zeba Desalt Spin Columns, 10 ml,** 25 each, for 700-4,000 µl samples
22360	**SMCC** (succinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate), 50 mg
22322	**Sulfo-SMCC** (sulfosuccinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate), 50 mg
22622	**Sulfo-SMCC, No-Weigh™ Format**, 8 × 2 mg microtubes
21555	**DSS** (disuccinimidyl suberate), 1 g
21655	**DSS,** 50 mg
21658	**DSS in No-Weigh™ Format,** 8 × 2 mg microtubes
21580	**BS^3 (bis[sulfosuccinimidyl] suberate),** 50 mg
21585	**BS^3 in No-Weigh™ Format,** 8 × 2 mg microtubes

References

1. Grabarek, Z. and Gergely, J. (1990). Zero-length crosslinking procedure with the use of active esters. *Anal Biochem* **185:**131-5.
2. Staros, J.V., *et al.* (1986). Enhancement by *N*-hydroxysulfosuccinimide of water-soluble carbodiimide-mediated coupling reactions. *Anal Biochem* **156:**220-2.
3. Timkovich, R. (1977). Detection of the stable addition of carbodiimide to proteins. *Anal Biochem* **79:**135-43.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermo.com/pierce