INSTRUCTIONS

Pierce Premium Grade EDC

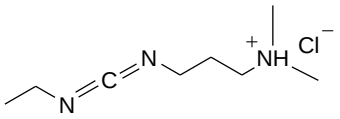
MAN0011875
Rev. B.0
Pub. Part No. 2162536.1

PG82079 PG82073 PG82074

Number	Description
PG82079	**Premium Grade EDC** (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride), 1g
PG82073	**Premium Grade EDC,** 25g
PG82074	**Premium Grade EDC,** 500g

Molecular Weight: 191.7

CAS # 25952-53-8



Storage: Upon receipt store product desiccated at -20°C. Product is shipped with an ice pack.

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

Introduction

Thermo Scientific™ Pierce™ Premium Grade Reagents are high-quality formulations of selected chemical modification reagents, specially characterized for applications where product integrity and risk minimization are critical. Compared to standard grade equivalents, Pierce Premium Grade Reagents provide more clearly defined quality and product support by including: (a) increased analytical testing and product characterization, (b) greater batch-specific information and quality assurance review, (c) extensive lot sample retention, and (d) change control notification.

Thermo Scientific™ Pierce™ Premium Grade 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 a urea by-product (see 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 (for larger-scale coupling, scale accordingly)

- 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 (e.g., Thermo Scientific, Product No. 28390)
- Pierce Premium Grade 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 Pierce Premium Grade 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.

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4. For BSA or OVA conjugation, dissolve 10mg of Pierce Premium Grade EDC in 1mL of ultrapure water and immediately add 100µL of this solution to the carrier-peptide solution. For KLH conjugation, dissolve 10mg of Pierce Premium Grade 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.

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

The following protocol, adapted from a procedure described by Grabarek and Gergely,[1] 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 (for larger scale coupling, scale accordingly)

- 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 (e.g., Thermo Scientific, Product No. 28372)
- Protein # 1: Prepared in Activation Buffer at 1mg/mL
- Protein # 2: Prepared in Coupling Buffer
- Pierce Premium Grade Sulfo-NHS (Product No. 82071 and 82072)
- 2-Mercaptoethanol (e.g., Thermo Scientific, Product No. 35600)
- Hydroxylamine•HCl (e.g., Thermo Scientific, Product No. 26103)
- (Optional) Zeba Spin Desalting Column (Product No. 89891) or other gel filtration column

Procedure

1. Equilibrate Pierce Premium Grade EDC and Pierce Premium Grade Sulfo-NHS to room temperature before opening bottles.
2. Add 0.4mg EDC (~2mM) and 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 non-reacted 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 a urea by-product (Figure 1).

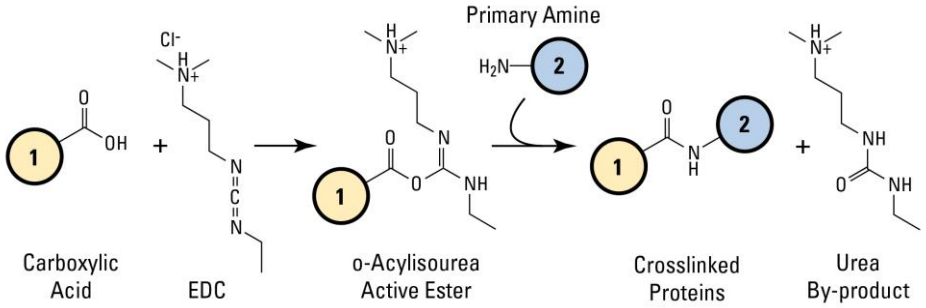

Figure 1. One-step EDC reaction with carboxyl- and amine-containing molecules.

Information On 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	**Pierce NHS** (N-hydroxysuccinimide), 25g
24510	**Pierce Sulfo-NHS** (N-hydroxysulfosuccinimide), 500mg
24525	**Pierce Sulfo-NHS,** 5g
24520	**Pierce Sulfo-NHS, No-Weigh™ Format,** 8 × 2mg microtubes
77149	**Pierce EDC,** 10mg
22980	**Pierce EDC,** 5g
22981	**Pierce EDC,** 25g
PG82071	**Pierce Premium Grade Sulfo-NHS,** 500mg
PG82072	**Pierce Premium Grade Sulfo-NHS,** 10g
20036	**Bioconjugate Techniques (Book),** 1202 pages, softcover
89889	**Zeba Spin Desalting Columns,** 2mL, 5 each, for 200-700µL samples
89891	**Zeba Spin Desalting Columns,** 5mL, 5 each, for 500-2000µL samples
89893	**Zeba Spin Desalting Columns,** 10mL, 5 each, for 700-4000µL samples
22360	**Pierce SMCC** (succinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate), 50mg
22322	**Pierce Sulfo-SMCC** (sulfosuccinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate), 50mg
22622	**Pierce Sulfo-SMCC, No-Weigh Format**, 8 × 2mg microtubes

PG82085	**Pierce Premium Grade Sulfo-SMCC,** 100mg
PG82086	**Pierce Premium Grade Sulfo-SMCC,** 1g
21555	**Pierce DSS** (disuccinimidyl suberate), 1g
21655	**Pierce DSS,** 50mg
21658	**Pierce DSS, No-Weigh Format,** 8 × 2mg microtubes
21580	**Pierce BS^3** (bis[sulfosuccinimidyl] suberate), 50mg
21585	**Pierce BS^3, No-Weigh Format,** 8 × 2mg microtubes
PG82083	**Pierce Premium Grade BS^3,** 100mg
PG82084	**Pierce Premium Grade BS^3,** 1g

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.