INSTRUCTIONS

Pierce EDC, No-Weigh Format

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A35391

Number	Description
A35391	**Pierce EDC, No-Weigh Format** (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride), 10 × 1mg Molecular Weight: 191.7 CAS # 25952-53-8

Storage: Upon receipt store product desiccated at -20°C. EDC is shipped on ice packs.

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

Introduction

Thermo Scientific™ Pierce™ 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 (NHS).[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]

Thermo Scientific™ No-Weigh™ products are specialty reagents provided in a pre-aliquoted format. The pre-weighed packaging prevents the loss of reagent reactivity and contamination over time by eliminating the repetitive opening and closing of the vial. The format enables use of a fresh vial of reagent each time, eliminating the hassle of weighing small amounts of reagents and reducing concerns over reagent stability.

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.

Additional Materials Required

- Activation Buffer: 0.1M MES, 0.5M NaCl, pH 6.0. Alternatively, use Thermo Scientific™ BupH™ MES Buffered Saline (Product No. 28390)
- Coupling Buffer: Phosphate-buffered saline (PBS), 100mM sodium phosphate, 150mM NaCl; pH 7.2 (Product No. 28372)
- Protein # 1: Prepared in Activation Buffer at 1-10mg/mL
- Protein # 2: Prepared in Coupling Buffer at 1-10mg/mL
- NHS, Sulfo-NHS, or Sulfo-NHS, No-weigh format (Product No. 24500, 24510, 24520 respectively)
- 2-Mercaptoethanol (Product No. 35600) for quenching the EDC activation reaction

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- (Optional) Thermo Scientific™ Zeba™ Spin Desalting Column (Product No. 89891) or other gel filtration column
- Hydroxylamine-HCl (Product No. 26103) for quenching the amine reaction
- Optional: Desalting columns (e.g. Thermo Scientific™ Zeba™ Spin Desalting columns.)

Procedure

1. Equilibrate the EDC, NHS, or Sulfo-NHS to room temperature before opening.
2. Prepare No-Weigh EDC by dissolving 1 vial (1mg) in 100µL of water or MES buffer to give a 50mM solution.

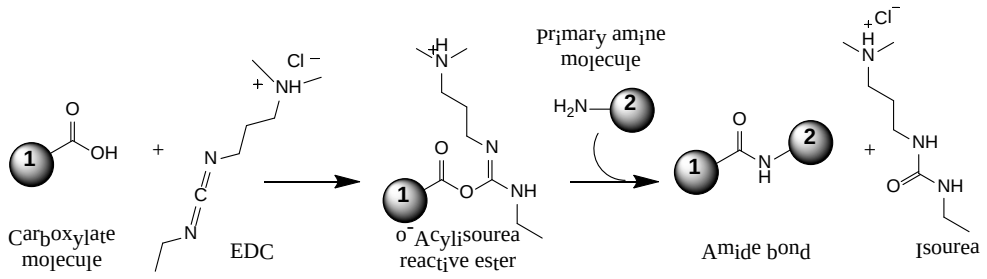
   Note: The maximum useable volume of each vial is 800µL.
3. Add 40µL EDC (prepared in step 2) and either 0.6mg of NHS or 1.1mg of sulfo-NHS to 1mL of Protein #1 solution. If using the No-Weigh format of Sulfo-NHS, add 40µL of water or Activation Buffer to a microtube for a 230mM solution. Add 22µL of the Sulfo-NHS solution to the 1mL of Protein #1 solution. React for 15 minutes at room temperature. Final concentrations of the reactants are 2mM (EDC) and 5mM (NHS/Sulfo-NHS).

   Note: For best results, use a 10-fold molar excess of EDC to Protein #1 and maintain a molar ratio of 1:2.5 of EDC:NHS. For a 50kDa protein at 10mg/mL, this results in a 10-fold molar excess of EDC to Protein #1
4. Add 1.4µL of 2-mercaptoethanol (final concentration of 20mM) to quench the EDC.

   Note: 2-mercaptoethanol may reduce disulfide bonds possibly present in Protein #1.
5. Optional: If it is desired to separate activated Protein #1 from excess EDC, EDC-byproduct, and NHS without using 2-mercaptoethanol, one may use an appropriate sized desalting column that has been equilibrated with PBS. Follow desalting column instructions and recover the fraction containing the activated protein. If using absorbance at 280nm to identify fractions containing protein, be aware that NHS and Sulfo-NHS absorb strongly at 260-280nm.
6. Add Protein #2 to the activated protein at an equal molar ratio with Protein #1. Mix well and allow components to react for 2 hours at room temperature.
7. 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.
8. 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.



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
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	**BS^3 (bis[sulfosuccinimidyl] suberate),** 50mg
21585	**BS^3, No-Weigh Format,** 8 × 2mg microtubes

General 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.

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