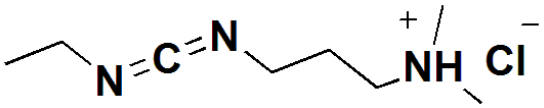
EDC-HCl Protocol and Product Information Sheet

Product Category:	Zero-Length Crosslinkers
Catalog Number(s):	c1100-3x10mg, c1100-100mg, c1100-5gm, c1100-25gm, c1100-100gm
Product Name:	EDC-HCl
Alternative Name(s):	EDC; EDAC; EDAC-HCl; 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide HCl
CAS Number:	25952-53-8
Chemical Formula:	$C_8H_{17}N_3$ HCl
Molecular Weight:	191.70
Spacer Arm Length:	N/A
Storage:	-20ºC or below (shipped at ambient temperature).



Background Information

Zero-length crosslinking allows scientists to immobilize protein-protein interactions without introducing a spacer arm. The EDC-HCl / Sulfo-NHS system has been perhaps the most successful way of creating zero-length crosslinks for decades. Facilitated through a reactive carbodiimide (EDC-HCl) and Sulfo-NHS as a catalyst, this coupling procedure is a highly efficient choice for crosslinking proteins or immobilizing proteins to a support. This procedure is designed to assist the scientist in crosslinking proteins or creating protein:protein conjugates. Immobilization techniques and other uses for this set of reagents can be obtained by contacting ProteoChem's technical support.

General EDC-HCl Protein Crosslinking Protocol

Important Notes:

- EDC-HCl is moisture sensitive. Allow EDC-HCl to equilibrate to room temperature before opening vial.
- Except as indicated in this protocol, avoid using buffers that contain DTT, EDTA, or β-Mercaptoethanol, as these can interfere with conjugation reaction.
- It is recommended that the incubation steps of this procedure be done using a rotary stirring device, although intermittent light vortexing is also acceptable.
- When choosing Protein #1 (**P_1**) and Protein #2 (**P_2**), ensure that **P_1** has free carboxyl groups available (COOH) and **P_2** has free amine groups available (NH_2).
  P_1: Terminal COOH, Asp, and Glu
  P_2: Terminal NH_2, Lys

Reagents and Buffers Needed

Conjugation Buffer: 100 mM MES (cr8107-25gm), 500 mM NaCl, pH 6.0
(Abr. MES = 2(N-morpholino)ethanesulfonic acid)
β-Mercaptoethanol
Sulfo-NHS (c1102-500mg)
Hydroxylamine-HCl (cr8108-25gm)
Desalting Column(s): Sephadex® G-25 (g4109) or equivalent

Step 1. Activation of Protein #1

a. Dissolve Protein #1 ($\mathbf{P_1}$) at 1-2 mg/mL in 1.0 mL Conjugation Buffer
Record $\mathbf{P_1}$ Concentration:
(mg $\mathbf{P_1}$) / [(MW protein in mg/mmol) * (mL Buffer)] = _____ mM $\mathbf{P_1}$

b. Weigh out 0.8 mg EDC-HCl and 2.2 mg of Sulfo-NHS and add directly to the protein solution in Step 1.a. This will give 4 mM EDC-HCl and 10 mM Sulfo-NHS.

c. Gently vortex reaction mixture until all reagent is soluble.

d. Allow Activation Reaction to proceed at room temperature for 15 minutes.

e. Add 1.4 µL of β-Mercaptoethanol to deactivate excess EDC-HCl.
Note: If either protein has critical disulfide bonds, this deactivation step can be omitted. In such a case, proceed directly to Step 2, or desalt activated ***P_1*** *through gel filtration, then proceed to Step 2.*

Step 2. Conjugation of Two Proteins

a. Dissolve Protein #2 ($\mathbf{P_2}$) in conjugation buffer at a concentration of 1-2 mg/mL. Ideally, this should be at the same concentration as $\mathbf{P_1}$.
Record $\mathbf{P_2}$ Concentration:
(mg $\mathbf{P_2}$) / [(MW protein in mg/mmol) * (mL Buffer)] = _____ mM $\mathbf{P_2}$

b. Gently vortex and allow the crosslinking reaction to proceed for 1.5 to 3 hours at room temperature. Use of rotary mixer is preferred.

c. Quench the conjugation reaction by adding hydroxylamine (to give a final concentration of 5 to 10 mM).

d. Purify the obtained conjugate from excess reagents and reaction by-products by gel filtration or dialysis. Exchange sample into buffer of choice. Conjugate bonds are stable under most biological conditions.

References:

Analytical Biochemistry 185, 131-135 (1990).
Anjaneyulu, P.S.R., Staros, J.V. (1987) J. Pept, Proteein Res. 30, 114-124.
Carraway, K.L., Triplett, R.B. (1970) Biochim. Biophys. Acta 200, 564-566.
Grabarek, Z., Gergerly, J. (1990) Anal. Biochem. 185, 131-135.
Hermanson, G.T. (2008) Bioconjugate Techniques, 2nd Ed. Academic Press, New York.

ProteoChem, Inc. * 7300 Edward Dr. * Loves Park, IL 61111
Toll Free: 888.501.2436 * Phone: +1.720.239.1630 * Fax: +1.720.239.1631
Technical Support E-mail: ts@proteochem.com
www.proteochem.com