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Technical Bulletin

Coupling Two Proteins with EDC-HCl and Sulfo-NHS

General Protein Coupling Protocol with EDC and Sulfo-NHS

1. To a 2 mL sample of protein #1(approx. 10 mg/mL, MW=60,000 Da) dissolved in reaction buffer (such as Phosphate buffered saline, pH 7.2), add 0.77 mg of EDC-HCl (4.0 µmol).
2. To this solution, add 2.17 mg of Sulfo-NHS (10.0 µmol), and mix well.
3. Incubate this reaction at room temperature for 10 to 15 minutes.
4. (Optional Steps) Deactivate residual EDC-HCl with excess 2-mercaptoethanol (2.5 µL). Separate the small molecule by-products (EDC, NHS, and 2-mercaptoethanol) via gel filtration to recover activated protein #1.
5. Prior to proceeding with the amine coupling portion of this reaction, ensure that the buffer pH is pH 7 to 8. Adjust the pH to within this range by adding sodium carbonate or concentrated phosphate buffer. Note: avoid any compounds or buffers containing amines, such as glycine or Tris.
6. Add Protein #2 lyophilized solid (or as 1-10 mg/mL solution in PBS or other suitable buffer) to the activated protein #1 solution . Mix. Allow reaction to proceed for 2 to 2.5 hours at room temperature.
7. (Optional) Quench the reaction with glycine, ethanolamine, hydroxylamine or other suitable amine at a final concentration of 10-20 mM.
8. (Optional) Remove unreacted small molecules via gel filtration with Sephadex G-25.

Notes: A good starting point for this coupling reaction is a molar ratio of Protein:EDC:NHS at approximately 1:10:25. This protocol reflects these ratios, although they are routinely modified and should be optimized on a case by case basis.

Related Products

Package Size and Product Numbers

EDC-HCl.....13405

Sulfo-NHS.....13505

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