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Covalent Coupling of Proteins to Carboxylated Polystyrene Microparticles by the "Carbodiimide" Method

Material

Material Required

- Stock Solution: Prepare using distilled or deionized water unless otherwise indicated.
- 0.1 M Carbonate Buffer: Prepare by adding 0.1 M Na_2CO_3 to 0.1 M $NaHCO_3$ until pH 9.6 is reached.
- 0.1 M MES Buffer: Dissolve 19.2g of MES free acid (MW 195.2) in ~900ml of pure water. Titrate to desired pH (5.2-6.0) with 1N NaOH. Make up volume to 1,000ml with pure water.
- 2% Carbodiimide: 2% 1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide hydochloride dissolved in MES Buffer. *Note:* use within 15 minutes of preparing.
- 0.2 M Borate Buffer: Prepare by adding 1 M NaOH to Boric acid until pH 8.5 is reached.
- 0.25 M Ethanolamine: Prepare by adding 20µl of ethanolamine (2-aminoethanol) to 1.3ml of Borate Buffer.
- Storage Buffer: 0.01 M phosphate buffer (pH 7.4), 1% BSA, 0.1% sodium azide, and 5% glycerol.
  Prepare 0.1 M stock of Sodium Phosphate Monobasic (NaH_2PO_4), 13.8 g/L, monohydrate (MW 138.0); and 0.1 M stock of Sodium Phosphate Dibasic (Na_2HPO_4), 26.8 g/L, heptahydrate (MW 268.0). Mix 19ml of the monobasic and 81ml of the dibasic to yield pH 7.4. Dilute this to a final volume of 1.0L after adding 1.0g of BSA, 5ml of glycerol, and 0.1g of sodium azide.

Procedure

Researchers are advised to optimize the use of particles in any application.

1. Place 0.5ml of 2.5% carboxylated microparticles into Eppendorf centrifuge tube (1.5-1.9ml capacity).
2. Add sufficient 0.1 M Carbonate Buffer to fill tube.
3. Centrifuge 5-6 minutes in a microcentrifuge.
4. Carefully remove supernatant using a Pasteur pipette. Discard supernatant.
5. Repeat steps 2, 3 and 4.
   Note: To resuspend pellet**:
   a) fill tube halfway and cap
   b) vortex
   c) fill tube to capacity
6. Resuspend pellet to one half the tube volume in 0.1 M MES Buffer.
7. Centrifuge for 5-6 minutes.
8. Carefully remove supernatant using a Pasteur pipette. Discard supernatant.
9. Repeat steps 6, 7 and 8, two times.
10. Resuspend pellet in 0.625ml of 0.1 M MES Buffer.
11. Add dropwise 0.625ml of 2% Carbodiimide.
12. Mix 3-4 hours at room temperature.
13. Centrifuge for 5-6 minutes. Remove and discard supernatant.
14. Resuspend pellet in 0.1 M MES Buffer.
15. Centrifuge for 5-6 minutes. Remove and discard supernatant.
16. Repeat steps 14 and 15, two times.These steps get rid of unreacted carbodiimide.
17. Resuspend pellet in 1.2ml of 0.2 M Borate Buffer.
18. Add 200-400µg of protein to couple (we have used rabbit anti-goat IgG, IgG fraction).
19. Mix gently overnight at room temperature on an end-to-end mixer.

20. Centrifuge for 10 minutes. Note the volume of the supernatant and save for protein determination. The amount of protein added in step 18 less the amount in the supernatant represents the amount bound to the beads.
21. Resuspend in 1.2ml of 0.2 M Borate Buffer. Add 50µl of 0.25 M Ethanolamine. Mix gently for 30 minutes. This step serves to block unreacted sites on the microparticles.
22. Centrifuge for 10 minutes. Remove and discard supernatant. Resuspend pellet in 1ml of 10mg/ml BSA solution in 0.2 M Borate Buffer. Cap and vortex.
23. Mix gently for 30 minutes at room temperature. This step will block any remaining non-specific protein binding sites.
24. Centrifuge for 5-6 minutes. Remove and discard supernatant.
25. Repeat steps 22 and 24.
26. Resuspend pellet in 0.5ml of Storage Buffer.

** When term “resuspend pellet” is used, please refer to step 5.

Notes

1 There are many variations used for this procedure. This protocol is offered as a guide and a convenience. Specific situations may require one or more alterations of this protocol. This procedure can be used for coupling proteins to research quantities of microparticles. To use this protocol on a larger scale, increase all volumes in a proportional manner.

2. This procedure is recommended for microspheres 0.5µm or larger. If using microspheres between 0.1µm and 0.5µm, please use our PolyLink Kit with Hollow Fiber Filtering System (catalog #24818). For microspheres less than 0.1µm, dialysis tubing should be used.

Storage and Stability

Store at 4°C. Freezing of particles may result in irreversible aggregation and loss of binding activity.

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