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Covalent Coupling of Proteins to Amino and Blue Dyed

*Polystyrene Microparticles by the "Glutaraldehyde" Method**

Note:
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.

Phosphate Buffer Saline:
(PBS), pH 7.4
First, prepare 0.1 M phosphate buffer, pH 7.4, by adding 0.1 M NaH_2PO_4 to 0.1 M Na_2HPO_4 until pH becomes 7.4. To make PBS, take 200ml of the 0.1 M phosphate buffer, pH 7.4, in a 1 liter volumetric flask. Add 8.77g of NaCl and make up the volume to one liter with DI water. Check the pH of the solution. If necessary, adjust the pH to 7.4 by using diluted HCl or NaOH.

0.5 M Ethanolamine:
Add 0.15ml of ethanolamine (2-aminoethanol) to 4.8ml of PBS, pH 7.4.

Storage Buffer:
Take 20ml of 0.1 M phosphate buffer, pH 7.4, in a 100ml graduated cylinder. Add 0.88g NaCl, 1g bovine serum album in (BSA), 5ml glycerol, and 0.1g NaN_3, and make up the volume to 100ml. Check the pH of the final solution. If necessary, adjust the pH to 7.4 by using diluted HCl or NaOH.

Procedure:
NOTE: Centrifuge speed time will vary with particle size.
1. Transfer 1ml of a 2.5% suspension of beads into an Eppendorf tube (1.5ml - 1.9ml capacity).
2. Fill the tube with phosphate buffered saline (PBS), pH 7.4, and cap the tube.
3. Centrifuge in a micro centrifuge until beads are pelleted.
4. Remove supernatant carefully using a Pasteur pipette. Discard supernatant.
5. Fill the tube with PBS, cap the tube, and resuspend the beads using a Vortex mixer.
6. Centrifuge until beads are pelleted.
7. Repeat steps 4, 5, and 6 twice.
8. Resuspend pellet in 1ml of 8% glutaraldehyde (EM Grade) in PBS, pH 7.4.
9. Leave overnight at room temperature with gentle end-to-end mixing.
10. Spin until beads are pelleted and remove supernatant
11. Wash the pellet three times with PBS (Steps 5 and 6).
12. Resuspend the washed beads in 1ml of PBS, pH 7.4, and add 400-500µg of protein.
13. Mix gently for 4-5 hours at room temperature by end-to-end mixing.
14. Spin until beads are pelleted and save supernatant for protein determination. The amount of protein added in Step 12 minus the amount in the supernatant represents the amount bound to the beads.
15. Resuspend pellet in 1ml of 0.5 M ethanolamine in PBS and mix for 30 minutes at room temperature by end-to-end mixing.
16. Spin until beads are pelleted and remove supernatant
17. Resuspend pellet in 1ml of 10mg/ml bovine serum albumin (BSA) in PBS.
18. Mix for 30 minutes at room temperature and spin. Discard supernatant
19. Resuspend pellet in 1ml of 10mg/ml BSA in PBS and spin.
20. Resuspend pellet in 1ml of PBS, pH 7.4, containing 10mg/ml BSA, 0.1% NaN_3 and 5% glycerol (storage buffer).

Store at 4°C. DO NOT FREEZE!

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*** This procedure is recommended for Microspheres 0.5µ or larger. If using Microspheres smaller than 0.5 microns, please use our Glutaraldehyde Kit with Hollow Fiber Filtering System (catalog #23964).**