

Corporate Headquarters 400 Valley Road Warrington, PA 18976 1-800-523-2575 FAX 1-800-343-3291 Email: info@polysciences.com www.polysciences.com

Europe - Germany Polysciences Europe GmbH Handelsstr. 3 D-69214 Eppelheim, Germany (49) 6221-765767 FAX (49) 6221-764620 Email: info@polysciences.de

TECHNICAL DATA SHEET 238D

Page 1 of 1

Covalent Coupling of Proteins to Amino and Blue Dyed

Polystyrene Microparticles by the "Glutaraldehyde" Method*

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 NaH₂PO₄ to 0.1 M Na₂HPO₄ 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 HCI 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 NaN₃, 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 HCI or NaOH.

Procedure:

NOTE: Centrifuge speed time will vary with particle size.

- 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.
- Centrifuge in a micro centrifuge until beads are pelleted.
- Remove supernatant carefully using a Pasteur pipette. Discard supernatant.
- Fill the tube with PBS, cap the tube, and resuspend the beads using a Vortex mixer.
- Centrifuge until beads are pelleted.
- Repeat steps 4, 5, and 6 twice.
- 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% NaN3 and 5% glycerol (storage buffer).

Store at 4°C. DO NOT FREEZE!

To Order:

In The U.S. Call: 1-800-523-2575 • 215-343-6484 In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767 In Germany FAX: (49) 6221-764620

Order online anytime at www.polysciences.com

* 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).

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation.

Data Sheet #238D

