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TECHNICAL DATA SHEET 238E

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Protocol for Adsorbing Proteins on Polystyrene Microparticles*

Note:

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.

Borate Buffer:

0.1 M Borate buffer, pH 8.5 - prepare a 0.1 M solution of boric acid and adjust pH to 8.5 with 1 M NaOH.

Storage Buffer:

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. Take 20ml of 0.1 M phosphate buffer, pH 7.4, in a 100ml graduated cylinder. Add 0.88g NaCl, 1g bovine serum albumin (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:

1. Take 0.5ml of a 2.5% suspension of the beads in an Eppendorf tube (1.5ml - 1.9ml capacity).
2. Fill the tube with 0.1 M borate buffer, pH 8.5, mix using Vortex mixer.
3. Centrifuge beads until pelleted.
4. Remove supernatant using a Pasteur pipette, fill the tube with borate buffer, resuspend the beads using a Vortex mixer, and spin for 5-6 minutes.
5. Repeat Step 3&4 twice.
6. Centrifuge beads until pelleted, remove supernatant and resuspend pellet in 1ml borate buffer.
7. Add 300-400 μg of the protein to be coupled, and incubate overnight at room temperature with gentle end-to-end mixing.
8. Spin for 10 minutes and save supernatant for protein determination. The amount of protein added in Step 6 minus the amount in the supernatant represents the amount bound to the beads.
9. Resuspend pellet in 1ml of 10mg/ml bovine serum albumin (BSA) in borate buffer.
10. Incubate for 30 minutes at room temperature with gentle mixing.
11. Spin for 5-6 minutes and remove supernatant.
12. Repeat steps 9 thru 11 twice.
13. 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!

*Not recommended for microparticles smaller than 0.5 μ .

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