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Protocol for Adsorbing Protein on Polystyrene Microspheres

INTRODUCTION

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. Please note that this procedure is not recommended for microspheres smaller than 0.5µm.

PROCEDURE

Researchers are advised to optimize the use of particles in any application, as procedures designed by other manufacturers may not be ideal.

0.1 M Borate Buffer, pH 8.5

Prepare a 0.1 M solution of boric acid and adjust to pH 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 for Adsorbing Protein

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, and 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 Steps 3 and 4, twice.
6. Centrifuge beads until pelleted, remove supernatant and resuspend pellet in 1ml borate buffer.
7. Add 300-400µg 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 7 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, 10 and 11, twice.
13. Resuspend pellet in 1ml of PBS, pH 7.4, containing 10 mg/ml BSA, 0.1% NaN_3 and 5% glycerol (Storage Buffer).

STORAGE

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

Products are for research use only and are not intended for use in humans or for *in vitro* diagnostic use.

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