ADSORBING PROTEIN ON POLYSTYRENE BEADS & BINDING ASSAY

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BACKGROUND

- This protocol is designed to assist with passive coupling of proteins to polystyrene microspheres. As per manufacturer's recommendations, this protocol is not advised for beads smaller than half of a micron.

NOTES

- The protein adsorption to beads should be done several days before the bead binding expt.
- Make sure to book the plate reader well in advance

EQUIPMENT

- Polysciences polystyrene microspheres
- 0.1M Borate buffer, pH 8.5
- Mal-BSA or protein desired to be coated on beads
- Cultured cells; if transiently transfected, use cells 48hr post-transfection; 1 million/tube needed (1-2 T150 flasks per cell type)
- Gibco Opti-mem media (Serum free complete; to ensure endocytosis is non opsonic)
- Vortex
- Eppendorf tubes & pipettes
- Centrifuge
- Sodium carbonate buffer, 0.2M pH 8.5
- 0.5M NaOH
- SDS PAGE Reagents
- Silver Stain (0.2% $AgNO_3$ + 0.028% Formaldehyde, 0.4mg sodium thiosulfate)
- Fixing solution (50% methanol, 40% ddH_2O, 10% acetic acid)
- 50% ethanol
- 0.02% sodium thiosulfate
- Developer solution (6% Na2CO3, 0.0185% formaldehyde, and 0.4 mg sodium thiosulfate)
- Plate Reader

PROTOCOL

PART I: Maleating BSA & SDS PAGE

1. Add BSA to 0.2M Sodium Carbonate buffer (pH=8.5) to a final concentration of 10mg/mL
2. Add Maleic anhydride to a final concentration of 0.1M
3. Adjust the pH to 7.5 or above using 0.5M NaOH
4. Prepare a 12% SDS PAGE Gel and load 7.5uL Kaleidoscope ladder (For preparation, see western blot procedure)
5. Load 500ug of sample and 500ug of un-Maleated BSA control and run the gel for 40 minutes at 200V
6. Soak gel in fixing solution for 1 hour with gentle rocking
7. Wash gel 2x with 50% ethanol with gentle rocking for 10 minutes
8. Soak the gel for 1 minute in 0.02% sodium thiosulfate solution in ddH_2O
9. Rinse the gel 3x for 30 seconds in ddH_2O
10. Add the silver nitrate solution for 10 minutes with gentle rocking
11. Rinse the gel 2x for 20 seconds in ddH_2O
12. Develop the band using developer solution with gentle rocking. Once the bands are bright enough, add the fixing solution.
13. Observe the gel; Mal-BSA should be larger in size than the control.

PART II: Adsorbtion of protein to polystyrene microspheres

1. Take 0.5mL of a 2.5% suspension of the beads in an eppendorf tube and fill with 0.1M borate buffer (pH = 8.5). Vortex the sample several times.
2. Centrifuge the beads at 12,000g (or higher) for 5 minutes. Larger beads require less speed.
3. Remove the supernatant and fill the tube with borate buffer and fill the tube with borate buffer and spin again for 5 minutes at 12,000g.
4. Remove the supernatant and resuspend the pellet in 1mL borate buffer.
5. Add 300-400ug of the protein to be coupled (in the case of mal-BSA; add 500uL), and incubate overnight at room temperature with gentle mixing.
6. Spin at 12,000g for 10 minutes and save the supernatant for protein determination. The amount of protein added previously minus the amount in the supernatant is representative of the amount of protein on the beads.
7. Resuspend in borate buffer and Incubate for 30 minutes at room temperature with gentle mixing. Spin at 12,000g for 5 minutes. Remove supernatant, resuspend in borate buffer and spin and remove supernatant again.
8. Remove the supernatant and resuspend the pellet in 1mL PBS. Store the beads at 4°C. DO NOT FREEZE.

PART III: Bead Uptake Assay

1. If using transiently transfected cells, use 48hr after transfection.
2. Remove old media and lift cells by blasting with Gibco Opti-mem serum free complete media.
3. Count the cells. 1 Million are required per tube in 1 mL of media.
4. The optimum MOI for beads is approx 320 beads/cell. This equates to 2uL of beads added to each tube. To avoid pipetting error, it is best to dilute these into 10uL stocks with 8uL PBS and add the entire 10uL portion.
5. Put cells on nutators at 4 and 37°C for 1.5hr.
6. Spin at 4°C 1500 rpm for 5 minutes for 4°C cells and 37°C for 37°C cells.
7. Remove the media, negative control pellets should be white (only cells) and positive will be yellowish (beads have been taken into the cells) and resuspend in 1mL PBS.

8. Spin at 1500 rpm for 5 minutes (room temperature is OK), remove PBS and resuspend in 1mL PBS. Repeat this one more time, but resuspend in 200uL PBS.
9. At this point, you can stain for specific receptors and run flow cytometry if desired.
10. Add 100uL of the resuspended cells into a 96 well plate and read at 441nm (If beads are FITC-conjugated).

LINKS AND REFERENCES

- Polysciences Technical Data Sheet 238E