PREPARATION OF PROTEIN-COATED POLYACRYLAMIDE BEADS

Materials

1. AOT (Bis(2-ethylhexyl)sulfosuccinate sodium salt, Fluka #86139), 100 mg.

2. Toulene.

3. Fluorescent dextran (>=500 kDa), 20 mg/ml in H2O.

4. Methanol, 100 ml.

5. PBS.

6. Acrylamide (40%, Bio-Rad) and Bis (2%, Bio-Rad).

7. Ammonium persulfate (Bio-Rad) solution, 10 mg in 100 ul distilled water. Prepare immediately before use in step 10.

8. TEMED (Bio-Rad).

9. MES buffer, 0.1 M, pH 4.9, 10 ml.

10. EDC, 26 mg/ml, prepare immediately before use.

11. Protein for coating, 10 mg/ml.

12. Acrylic acid (Fluka), comes as liquid.

Procedure (unless specified, all the steps are to be performed at room temperature)

1. Fit a 15 ml corex tube with a rubber stopper with a yellow pipette tip inserted through a hole. Place a flea size stir bar in the tube and clamp onto a ring stand in a hood. Set up a stirring plate under the tube and connect the stopper to the source of nitrogen gas.

2. Tare the corex tube w/o stopper and add AOT 40-60 mg. Add toulene to make a final concentration of 10 mg/ml AOT. Stir to dissolve.

3. Mix 5 ml of acrylamide solution in a small beaker according to the dilution scheme below.

Final Acrylamide/Bis	40%Acrylamide	2%Bis	1M HEPES	H_20+Other
8%/0.1%	1000 ul	250 ul	50 ul	3700 ul
8/0.08	1000	200	50	3750
8/0.06	1000	150	50	3800
8/0.05	1000	125	50	3825
8/0.04	1000	100	50	3850
8/0.03	1000	75	50	3875
8/0.02	1000	50	50	3900
5/0.12	625	300	50	4025
5/0.10	625	250	50	4075
5/0.08	625	200	50	4125
5/0.06	625	150	50	4175
5/0.05	625	125	50	4200
5/0.025	625	63	50	4262
3/0.10	375	250	50	4325

4. Add 200 ul of the FITC dextran stock and 10 ul of acrylic acid. Degas 30 minutes.

5. Add 30 ul ammonium persulfate, 20 ul TEMED. Mix gently by gentle swirling.

6. Immediately add 100 ul of the acrylamide mixture per ml of AOT while stirring. Allow acrylamide to polymerize for 1 hour while gently stirring (setting 2) under a gentle stream of nitrogen, which should barely move the surface of the liquid.

7. Spin in the Sorvall at 500 rpm for 5 minutes and remove the supernatant. Add 8-10 ml methanol. The beads should remain insoluble. Repeat the spin and resuspension procedure with MeOH 3-5 times, then with PBS for 3-5 times and resuspend the final pellet in PBS. The beads may be stored at 4^oC indefinitely.

8. Take 100 ul of stock bead solution, add 400 ul of MES buffer. Spin at 2,000 rpm for 2 minutes in an Eppendorf microfuge, collect the supernatent in a fresh tube and spin at 14,000 rpm for 2 minutes to collect the beads that are 1-10 microns in diameter.

9. Spin and resuspend the beads 3 times with 1 ml MES buffer for each wash in the Eppendorf microfuge as above.

10. Add 0.5 ml of 26 mg/ml EDC in MES, shake on a rocker for 2 hours.

11. Spin and resuspend the beads 3 times with MES buffer as in step 9.

12. Add 1 ml of the protein for coating and mix on a rocker overnight.

13. Spin and resuspend the beads 3 times with 1 ml PBS for each wash. Beads may be stored at 4^{o}C for up to 2 weeks, depending on the longevity of the coated proteins.

Note

The rigidity of these free-floating particles is likely lower than that of tethered thin sheets of the same acrylamide/Bis composition, due to the freedom to swell.

Reference

Beningo, K.A. and Wang, Y.-L. (2002) Fc-receptor mediated phagocytosis is regulated by mechanical properties of the target. *J. Cell Sci.* 115:849-856.