PROTOCOL FOR COUPLING ANTIBODIES TO POLYSTYRENE MICROSPHERES

Prepare in advance:

- MES buffer: 100 mM MES (2-(N-Morpholino)ethanesulfonic acid), pH=6
- Glycine buffer: 100 mM Glycine, adjust pH=6 with diluted NaOH and HCl
- Pluronic F127: 10 % Pluronic F-127 in milliQ H_2O, filter in steriflip

- Polystyrene microspheres (1 um diameter), amino-modified, fluorescent:
  SigmaAldrich L2778 (1um, NH_2, red fluorescent)
  SigmaAldrich L1030 (1um, NH_2, yellow-green fluorescent)

- EDC: *N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (Sigma). Store tightly wrapped at -20C

1. Dilute 25 ul microspheres in 1 ml MES buffer

2. Sonicate 2 min and centrifuge 5 min at 10,000 g

3. Resuspend in 800 ul MES buffer and vortex well

4. Add 10-50 µg antibody in no more than 100 µl water or a suitable buffer without free amino groups (make sure the Ab is not in Tris buffer!!). As negative control, use 50 µg bovine serum albumin prepared at 0.5 mg/ml in H_2O.

5. Prepare fresh EDC (Dimethylaminopropyl-3-ethylcarbodiimide) only at this point!
   10 mg EDC in 1 ml MES (votex well)

6. Add 100 µl EDC to the beads-Ab mixture and agitate well.

7. Keep at room temperature x 2 hours with constant rotation. Agitate and sonicate briefly every 20 min.

8. Quench the reaction with 350 ul glycine buffer, 30 min x room temperature

9. Centrifuge 5 min at 10,000 g

10. Resuspend in 1 ml PBS with 0.2 % Pluronic F127. Mix well until the suspension is homogeneous.

11. Centrifuge 5 min at 10,000 g. Repeat steps 10-11 again.

12. Resuspend in 1 ml PBS containing 0.2 % Pluronic F127 and 0.1 % BSA

13. Sonicate x 2 min.

14. Store in aliquots at 4C, wrapped in aluminum foil.

15. Use within two weeks (dilution 1/20 – 1/200 to detect surface antigens in cultured cells).

Bibliography: Riccio et. al. (1997) Science 277, 1097
Heerssen et. al. (2004) Nature Neuroscience 7, 596