Preparation of Protein-A – anti-DIG-coated latex beads

Type of bead/diameter:
Antibody used: Anti-Digoxigenin, polyclonal, Roche Cat. No. 1 333 089

I. Solutions:

1. Bead suspension:
3.18 µm diameter carboxylated latex beads (Spherotech), 5 %.

2. Protein-A *5* mg/ml
Dissolve Protein-A (Sigma) in H_2O at a final concentrationof 5 mg/ml.
Prepare 50-µl aliquots.
Quick-freeze aliquots and store at -80 ˚C.

3. Na-phosphate	100 ml
NaH_2PO_4 (monobasic) 0.1 M, pH 5	1.38 g

This monobasic solution will have a pH of ~5.

4. Carbodiimide (EDC or EDAC)
10 mg/ml in Na-phosphate
Prepare at least 1 ml.

5. Borate	100 ml	200 ml
0.1 M Na-borate	3.814 g	7.628 g

Adjust to pH 8.2

6 Triethanolamine	100 ml	200 ml
0.2 M Triethanolamine	3.714 g	7.428 g

Adjust to pH 8.2

7. DMP (dimethylpimelimidate)	10 ml	20 ml
50 mM DMP	0.13 g	0.26 g

Always make fresh.
Dissolve in Triethanolamine
Readjust pH to 8.2.

8. Ethanolamine	10 ml	100 ml	200 ml
50 mM Ethanolamine	31 µl	310 µl	620 µl

Concentration should equal that of DMP.
Dissolve inH_2O
Adjust pH to 8.2.

9. AB buffer Volumes:	100 ml	250 ml	1000 ml
25 mM imidazole-HCI (pH 7.4)	0.17 g	0.425 g	1.7 g
25 mM KCI	0.186 g	0.466 *g*	1.864 g
4 mM $MgCl_2$ (1 M stock)	0.4 ml	1 ml	4 ml
1 mM EGTA (0.5 M stock)	0.2 ml	0.5 ml	2 ml
1 mM DTT (add fresh from 1 M stock)	100 µl	250 µl	0.154 g

10. 0.2 M KCl/AB Volumes:	50 ml	100 ml
0.2 M KCl	0.7456 g	1.4914 g

-add AB buffer-

11. Bead blocking solution: Volume:	1 ml
1% BSA	50 µl stock (20%)
0.2 % Tween-20	20 µl stock (10%)

12. PBS	1 liter
10 mM K_2HPO_4/KH_2PO_4, pH 7.4	
140 mM NaCl	8.18 g

Kellermayer. latex bead coating. Date:
Number:

Preparation suggestions:
a) Prepare 500 ml 20 mM K-phosphate buffer from mono- and dibasic (pH 7.4). b) Add 8.18 g NaCl to 500 ml 20 mM K-phosphate solution, then bring volume to 1 liter with dH_2O.

II. Steps for protein-A – anti-digoxigenin beads:

A. Dissolution of Anti-Digoxigenin
1. Add 0.4 ml PBS to antibody liophilizate
2. Final antibody concentration is 0.5 mg/ml

B. Preparation of Protein-A-conjugated latex beads (reduce quantities as necessary)
1. Mix: -100 µl Protein-A
   -1 ml Bead suspension
   -1 ml EDC
2. Vortex
3. Rotorack for 2 hours at room temperature.
4. Centrifuge in tabletop centrifuge, 3000 rpm, 4 ˚C, 4 min.
5. Resuspend pellet in 1 ml 0.2 M KCl AB
6. Repeat steps 4-5 twice. Final bead concentration is 5%.

C. Conjugation of Anti-Digoxigenin antibody to Protein-A beads. (reduce quantities as necessary)
1. Centrifuge 400 µ1 Protein-A beads in tabletop centrifuge, 3000 rpm, 4 ˚C, 5 min.
2. Resuspend pellet in 400 µl Borate.
3. Repeat steps 1-2 twice.
4. Add 300 µl ANTI-DIG to the Protein-A bead susedsion.
5. Rotorack for 30 minutes at room temperature.
6. Centrifuge at 3000 rpm, 4 ˚C, 5 min.
7. Resuspend pellet in 800 µl borate.
8 Centrifuge at 3000 rpm, 4 ˚C, 5 min.
9. Resuspend pellet in 800 µl Triethanolamine.

D. Croslinking of anti-digoxigenin to Protein-A. (reduce quantities as necessary)
1. Centrifuge ANTI-DIG-conjugated beads at 3000 rpm, 4 ˚C, 5 min.
2. Resuspend pellet in 800 pl Triethanolamine.
3. Repeat steps 1-2.
4. Centrifuge at 3000 rpm, 4 ˚C, 5 min.
5. Resuspend pellet in 3 ml DMP. Prevortex pellet, then add DMP while vortexing.
6. Rotorack for 45 minutes at room temperature.
7. Centrifuge at 3000 rpm, 4 ˚C, 5 min.
8. Resuspend pellet in 800 µl Ethanolamine.
9. Rotorack for 5 minutes at room temperature.
10. Centrifuge at 3000 rpm, 4 ˚C, 5 min.
11. Resuspend pellet in 800 µl Borate.
12. Centrifuge at 3000 rpm. 4 ˚C, 5 min.
13. Resuspend pellet in 800 µl 0.2 M KCI-AB.
14. Centriguge at 3000 rpm, 4 ˚C, 5 min.
15. Repeat steps 13-14.
16. Resuspend pellet in 400 µl 0.2 M KCI-AB. Final bead concentration is ~5 %.

III. Steps for direct bead labeling with anti-digoxigenin:
1. Mix: -100 µl anti-dig
   -0.1 ml Bead suspension
   -0.1 ml EDC
2. Vortex
3. Rotorack for 2 hours at room temperature.
4. Centrifuge in tabletop centrifuge, 3000 rpm, 4 ˚C, 4 min.
5. Resuspend pellet in 1 ml 0.2 M KCl AB
6. Repeat steps 4-5 twice. Final bead concentration is 5%.