Preparation of Protein-A-antibody-coated latex beads

Type of bead/diameter:
Antibody used:

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%)

II. Steps:

A. Preparation of Protein-A-conjugated latex beads:
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 new IEC fuge, 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%.

B. Conjugation of T12 antibody to Protein-A beads.
1. Centrifuge 400 µ1 Protein-A beads in new IEC, 3000 rpm, 4 ˚C, 5 min.
2. Resuspend pellet in 400 µl Borate.
3. Repeat steps 1-2 twice.
4. Add 300 µl T12 (from Fürst) to the Protein-A bead suspedsion.
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.

C.Croslinking of T12 to Protein-A.
1. Centrifuge T12-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 %.

D. Pre-blocking T12 beads:
1. Mix 100 µl T12-bead suspension and 400 µl Bead-blocking solution.
2. Rotorack at room temp. for 5 min, vortexing 5s every minute.
3. Centrifuge in new IEC at 3000 rpm, 5 min.
4. Resuspend in 500 µl 0.2M KCl AB
5. Repeat steps 3-4.

E. Coating T12 beads with titin
1. Mix 50 µl pre-blocked T12 bead suspension, 5-15 µl titin, and 0.2M KCl AB (to 1 ml).
2. Rotorack at room temp for 30 min.
3. Centrifuge in new IEC at 3000 rpm, 5 min.
4. Resuspend in 1 ml 0.2 M KCl AB. Gently!