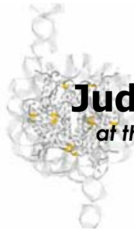

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Batch Crosslinking of Antibody to Protein G Sepharose

Revised by Rice Lab
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Procedure

- Warm bottle of DMP to room temperature before opening.
- Determine protein concentration of antibody solution, if necessary (i.e., ascites, supernatant).
- This method is easily scaled down 10-20 fold and done in microfuge tubes.
- Use different buffers with protein G-sepharose or protein A-sepharose (see below).

1. Place slurry of beads in a 15 ml red cap tube.
   Plan for 1 ml of packed protein G-Sepharose beads per antibody.

2. Spin for 5 minutes in IEC centrifuge at 2000 rpm at 4°C.

3. Aspirate supernatant to obtain 1 ml of packed beads.
   Do not let the beads become dry; leave a small volume of buffer over beads.

4. Begin with a volume that contains 2-5 mg of antibody.
   For example, ascites is ~20-25 mg/ml total protein, of which ~10-20% is IgG (2-5 mg/ml IgG), so try 1 ml ascites fluid.

5. Dilute to 9 ml with Borate buffer.
   - If using protein A-Sepharose beads, dilute antibody in 9 mls Borate/NaCl buffer. In all steps below use Borate/NaCl buffer in place of Borate buffer.

6. Spin for 10 minutes in IEC centrifuge at 2000 rpm at 4°C.
   Place on ice.

7. Reserving 100 µl of supernatant on ice for analysis by SDS-PAGE, transfer antibody solution (supernatant) to 15 ml red cap tube containing 1 ml packed beads.

8. Mix on rotator at room temperature for 30 minutes to 1 hour to bind antibody to Sepharose.

9. Spin beads 5 minutes in IEC centrifuge at 2000 rpm at 4°C.
   Save supernatant at 4°C to analyze later.

10. Resuspend the beads in 10 mls of Borate buffer.
    Rotate for 2 minutes at room temperature to wash beads.

11. Spin down beads for 5 minutes in IEC centrifuge at 2000 rpm at 4°C.

12. Repeat the wash steps (10-11) once more.
    Then, reserve 10 µl of packed beads in a microfuge tube for later analysis.

13. Add 9 ml of Borate buffer to 1 ml of beads.

14. Add 50 mg of DMP (dimethyl pimelimidate HCl) to bring final concentration to 20 mM.
    Warm DMP to room temperature before opening.

15. Mix on rotator for 30 minutes at room temperature.

16. Spin down beads for 5 minutes in IEC centrifuge at 2000 rpm at 4°C.

17. Resuspend the beads in 10 ml Ethanolamine buffer.
    Rotate for 5 minutes at room temperature to stop reaction.

18. Spin down beads for 5 minutes in IEC centrifuge at 2000 rpm at 4°C.

19. Resuspend in 10 ml Ethanolamine buffer.

20. Rotate for 1-2 hours at room temperature to quench unreacted DMP.

21. Spin down beads for 5 minutes in IEC centrifuge at 2000 rpm at 4°C.

22. Wash beads with 10 ml of PBS.
    Transfer beads to microfuge tube with PBS, and spin 10 seconds to pellet beads.
    Add to beads an equal volume of PBS + merthiolate.
    Dispense beads as 50% slurry for experiments.
    Beads are stable for more than one year at 4°C.

Verify Binding by SDS-PAGE

23. Mix 1 µl of supernatants from steps 7 and 9 with 9 µl of SDS-PAGE SB1.
    23.1. If antibody concentration is low, TCA precipitate 10 µl, and resuspend in SDS-PAGE SB2.
24. To 10 µl of packed beads from steps 12 and 22, remove excess buffer with pipette tip, and add 15 µl of SB1.

25. Boil samples for 5 minutes, spin down samples with beads, and run on minigel.

26. Stain with Coomassie Blue.
    Heavy chain bands (~55 kDal) should be visible in samples from steps 7 and 9, but not in 12 and 22. If uncoupled heavy chains are seen, wash beads with 100 mM glycine, pH 3.0.

Solutions

Borate buffer

200 mM sodium tetraborate decahydrate
pH 9.0

- Borate/NaCl buffer:
  Add 3 M NaCl to above,
  adjust to pH 9.0.

PBS + Merthiolate

PBS + 1/100 dilution of 1% merthiolate
(ethylmercurithiosalicyclic acid)

Ethanolamine buffer

200 mM ethanolamine, pH 8.0