Affinity purification of antibodies

Additional materials

1. Disposable Column: choose a column size appropriate for the volume of SulfoLink Coupling Gel (Pierce) to be used.
2. Disposable Column Trial Pack (Product No. 29925) contains accessories plus 2 each of three different sized columns, appropriate for 1–10 ml gel bed volumes.
3. Coupling Buffer: 50 mM Tris, 5 mM EDTA-Na, pH 8.5. If not already prepared for the Reduction Procedure, prepare a volume of buffer equal to 20 times the volume of SulfoLink Coupling Gel to be used.
4. L-Cysteine.HCl: Product No. 44889,
5. Wash Solution: 1 M sodium chloride,
6. Storage Buffer: phosphate buffered saline (PBS) or other suitable buffer containing 0.05% sodium azide,

Coupling protein/peptide to gel

1. Equilibrate SulfoLink Coupling Gel to room temperature (RT) and add an appropriate volume of gel slurry to an empty disposable column. For example, add 2 ml of gel slurry to obtain a gel bed of 1 ml.
2. Wash column with 4 volumes of Coupling Buffer. Throughout the entire procedure, do not allow the gel bed to run dry; add additional solution or replace the bottom cap on the column whenever the buffer drains to the top of the gel bed.
3. Replace bottom cap and add the sample prepared in the Reduction Procedure to the column, or dissolve the sulfhydryl-containing peptide in Coupling Buffer and add it to the column. Use 1 ml of protein solution per ml of SulfoLink Coupling Gel.
4. Replace the top cap and mix column (by rocking or end-over-end mixing) at RT for 15 min.
5. Incubate the column at RT for an additional 30 minutes without mixing.
6. Sequentially remove top and bottom column caps and allow the solution to drain from the column into a clean tube.
7. Place column over a new collection tube and wash with 3 volumes of Coupling Buffer.

Blocking nonspecific binding sites on gel

1. Replace the bottom cap on column.
2. Prepare a solution of 50 mM L-Cysteine.HCl in Coupling Buffer.
3. Apply 1 ml of 50 mM cysteine solution to the column for each ml of gel.
4. Replace the top cap and mix for 15 minutes at RT, then incubate the reaction without mixing for an additional 30 minutes at RT.

Washing the Column

1. Sequentially remove the top and bottom caps and allow the buffer to drain from the column.
2. Wash the column with at least 6 volumes of Wash Solution (1 M NaCl).
3. Wash the column with 2 column volumes of degassed Storage Buffer.
4. Replace the bottom cap and add an additional 2 ml of Storage Buffer.

Affinity purification (adapted form de Lange lab protocol)

1. The rabbit sera come from the company as 15–20 ml per bleed in plastic bottles. Store these bottles the way they come at –70°C until they are thawed for purification. The first time a bottle is opened, thaw it at room temperature or at 4°C overnight. Divide the serum into 2–5 ml aliquots, depending on strength of immune response. Clearly label the date of the bleed, use 2–5 ml per purification and store the rest at –70°C.
2. Wash affinity the affinity column with 30 ml of PBS.
3. Spin 2–5 ml of serum at max speed at 4°C and add it to the affinity column in cold room.
4. Collect the flow-through.
5. Pass the flow-through through the column four more times, save the final flow-through and store it at –70°C until you know the purification is successful, then the flow-through can be discarded. Subsequent steps can be done at room temperature.
6. Wash the column with 40 ml PBS (if antibody shows unspecific binding, washes can be performed under more stringent conditions by adding an additional washing step with higher salt concentration (e.g. 40 ml PBS, 40 ml PBS/0.5 M NaCl, 40 ml PBS)
7. Set up 8 Eppendorf tubes each containing 65 μl 1 M Tris pH 9.5; label them 1-8.
8. Elute antibody with 8 ml of 0.1 M Glycine pH 2.5; hand-collect 1-ml fractions into the Eppendorf tubes containing the Tris buffer and vortex immediately.
9. Measure protein content in each tube by Bradford using 20 μl eluant form each fraction (no need to set up a BSA standard in this step.)
10. Combine all fractions with OD_{595}>0.1 (usually the first 3–4 fractions)
11. Dialyze the antibody against cold PBS overnight in the cold room.
12. Measure the antibody concentration using Bradford method against BSA standards. The antibody concentration usually varies from 0.1 to over 1mg/ml. If the antibody is very dilute (e.g. < 0.1 mg/ml, add BSA to 1 mg/ml for stabilization.
13. Add sodium azide to 0.02%; aliquot and freeze at –70°C.
14. Wash column with 20 ml of PBS then with 10 ml PBS/0.02% sodium azide and store sealed at 4°C.