

AFFINITY-PURIFICATION OF ANTIBODIES VIA PROTEIN- COUPLED SEPHAROSE

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1. Preparation of affinity matrix and coupling of protein to the Sepharose matrix:

- a. Dialyze 1 mg of fusion protein in coupling buffer overnight at 4°C.
- b. Calculate the amount of cyanogen bromide (CNBr)-activated Sepharose4B needed for protein coupling. Usually, 1 g of CNBr activates 3.5 ml of Sepharose beads and 1 ml of activated Sepharose beads may absorb 5–10 mg of protein.
- c. Activate the Sepharose beads in 20–50 ml cold 1 mM HCl for 15 min at 4°C.
- d. Wash the beads with 1 mM HCl. In general, 1 g of Sepharose beads requires 200 ml of HCl to wash.
- e. Incubate appropriate amounts of activated Sepharose beads with dialyzed fusion proteins for 2 hrs at room temperature or overnight at 4°C.
- f. Wash protein-coupled Sepharose matrix with 15ml of coupling buffer.
- g. Add 5 ml 0.1 M Tris-HCl buffer (pH 8.0) or 1 M Ethanolamine to block the uncoupled sites on beads and let stand for 2 h at room temperature or overnight at 4°C.

2. Purification of antiserum via protein-coupled Sepharose:

- a. Wash the beads at least three cycles with acid and alkali buffer alternative. (0.1 M Acetic/Sodium Acetate, 0.5 M NaCl, pH4.0; 0.1 M Tris-HCl, 0.5 NaCl, pH 8.0).
- b. Incubate the beads with serum for 1–2 h at room temperature or overnight at 4°C.
- c. Collect the flow-through from the purification column and save it for ELISA testing.
- d. Wash the beads 3 times with 10 ml PBS buffer.
- e. Wash the column with 10 ml 150 mM NaCl-HCl (pH 5) solution.
- f. Elute the antibodies with 6 ml elution buffer and neutralize the solution with saturated phosphate buffer. Usually, 1 ml of elution buffer requires 50–100 µl of saturated phosphate buffer depending on the temperature.
- g. For short-term storage, keep the antibody solution at 4°C; for long-term storage, keep the antibody in a 50% glycerol solution with 0.02% sodium azide at -20°C.

3. Beads washing and recycling:

- a. Wash beads 3 times with 15 ml 0.01 M Tris-HCl (pH 7.5) buffer.
- b. Wash beads 3 times with 10 ml of PBS buffer.
- c. Add 2 ml PBS, 3 ml Glycerol with 0.02% sodium azide to the beads and store at -20°C for future use.

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Buffers Needed

Coupling Buffer	1000 ml
100 mM NaHCO ₃	8.40 g
500 mM NaCl	29.2 g
Add ddH ₂ O to 1000 ml	
Adjust to pH 8.3	
PBS Buffer	1000 ml
10 mM Na ₂ HPO ₄	1.42 g
1.8 mM KH ₂ PO ₄	0.24 g
137 mM NaCl	8 g
2.7 mM KCl	0.2 g
Add ddH ₂ O to 1000 ml	
Adjust to pH 7.4	
Elution Buffer	1000 ml
150 mM NaCl	8.8 g
Add ddH ₂ O to 1000 ml	
Use HCl to adjust to pH 2.5	
Saturated Phosphate Buffer	
Add Na ₂ HPO ₄ to PBS buffer until saturation.	