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# 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 Ethanolaniba 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 $ddH_2O$ to 1000 ml | |
| Adjust to pH 8.3 | |

| PBS Buffer | 1000 ml |
|---|---|
| 10 mM $Na_2HPO_4$ | 1.42 g |
| 1.8 mM $NaH_2PO_4$ | 0.22 g |
| 140 mM NaCl | 8.19 g |
| Add $ddH_2O$ to 1000 ml | |
| Adjust to pH 7.4 | |

| Elution Buffer | 1000 ml |
|---|---|
| 150 mM NaCl | 8.8 g |
| Add $ddH_2O$ to 1000 ml | |
| Use HCl to adjust to pH 2.5 | |

| Saturated Phosphate Buffer |
|---|
| Add $Na_2HPO_4$ to PBS buffer until saturation. |

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