Antibody affinity purification

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DO NOT LET THE COLUMN RUN DRY!!!!!

0. Spin 2ml serum in microcentrifuge, top speed, 10 minutes (in two eppendorf tubes).
1. Dilute 1 to 5 with 10 mM Tris pH7.5 and move to 15 mL conical. Place on ice.
2. Drain column.
3. Equilibrate column with 5 column volumes of 10 mM Tris pH7.5.
4. Apply diluted serum to column, collect flowthrough, reapply 3 times.
5. Wash column;
   20 mL (4x 5 mL)10 mM Tris pH7.5. and then with
   20 mL (4x 5 mL) 10 mM Tris pH7.5, 500 mM NaCl
6. Acid elution:
   with 10 mL 100 mM Glycine pH2-2.5 into 15 ml conical containing 1 ml 1M Tris pH 8.5
7. When done, immediately invert tube and place on ice. Check pH. (If less than 7. Add more 1 M Tris pH 7.5).
8. Reequilibrate column with 10 mM Tris pH7.5. Check pH of flowthrough, until it is 7.5.
9. Elute with base: with 100 mM triethylamine pH11.5 into 1 mL of 1 M Tris pH7.5.
10. When done, immediately invert tube and place on ice. Check pH. (If more than 7.5, add more 1 M Tris pH 7.5).
11. Reequilibrate column with 10 mM Tris pH7.5. Check pH of flowthrough, until it is 7.5.
12. Wash column with PBS. Store in PBS with 0.02% azide. Parafilm column and put back in the fridge.
13. Concentrate antibody.
    30 K Biomax centrifugal concentrator
    Prespin with 1 mL PBS.
    Add antibody, 2800 rpm, 15 min, 4°C
    Add 5 mL PBS, spin.
    Add 5 mL PBS, spin. With 200 uL pipette tip, move Ab to eppendorf tube.
    Store Ab at 4°C.
14. Take OD280
    OD 1 = 0.75 mg/mL