Purification of mAb (IgG)

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Purpose

Materials

- Antibody **7E3**, 2L sup grown in flasks, frozen and thawed overnight.
- BioRad Affi-Gel Protein A MAPS II Buffers cat. #1530-6160 ($161.00)
- 50 mM Tris pH7.8
- 40 g ammonium sulfate for every 100 ml Sup.
- 4L 100 mM Tris/pH7.8 or Binding Buffer
- BD tubing 14-170-12F (Fisher)
- Polyethylene tubing Clay Adanes.
- 18 Gauge needles.
- Binding Buffer (BioRad): make 1L. 314g/L ddH_2O and filter through 0.22 um, filter. pH=9.0
- Elution buffer (BioRad): make 500 ml. 11g/500 ml ddH_2O and filter as before. pH=3.0
- Regeneration buffer (BioRad): BioRad Affi-gel regeneration buffer.

Procedure

For Ammonium sulfate cut:

1. Filter Sup. in 1L Costar Filter using pre-filter.
2. Add 50 mM Tris pH7.8 (50 ml of 1 M stock/Liter). *100ml*
3. Add 40 g ammonium sulfate for every 100 ml Sup. (slowly). *800g*
4. Stir O/N in cold room.
5. Pour into plastic bottles. Spin at 4 ºC, 7,500 rpm for 20 min in JA-10 rotor.
6. Prepare 4L 100 mM Tris/pH7.8 (400 ml of 1 M stock/4 L) or Binding buffer.
7. Discard Sup. Resuspend pellets in 10 ml of 100 mM Tris buffer or Binding buffer. And pool into 50 ml Falcon tubes (try not to make bubbles).
8. Use 3.2 ml/cm 12-14,000 MW dialysis tubing.
    - Heat tubing in 500 ml H_2O in microwave. Not boiling.
    - Rinse tubing in fresh H_2O several times.
    - Test each tube w/H_2O and discard.
9. Add protein mixture to dialysis tube. Stir slowly in 100 mM Tris buffer or Binding buffer until pink color is out.
10. Transfer protein mixture (Ab) to 50 ml Falcon tubes to determine volume.
11. Dilute the mixture 1:1 with Binding buffer.
12. Filter through 0.45 um filters (use prefilters).

For Purification of Abs by Protein A column:

1. Prepare Protein A column.
2. Run binding buffer (pH9.0) ~ 200 ml.
3. Filter protein mixture
4. Add protein mixture or culture supernatant containing Ab (adjust pH to 7.8 with Binding buffer; red color) to the Protein A column.

Mouse antibodies of the IgG1 subclass do not have a high affinity for protein A. Purification on protein A beads using standard conditions will yield approximately 1/10 the amount of antibody compared with other subclasses. In case of IgG1 subclass, add 3.3 M NaCl (192.85 g/L) to crude antibody preparation (serum, tissue culture supernatant, or ascites).

5. Apply Binding buffer again ~ 200 ml.
6. Apply Elution buffer ~ 100 ml.
7. Collect 3 ml fractions in 5 ml tubes with 700 ul 1 M Tris pH 9.0 already in bottom of tube to neutralize (collect at ~5 min/fraction).
8. 25 tubes are sufficient for collection. In general, you can see high Ab concentrations in 7-8 tubes.
9. Test 1 ul on pH paper.
10. Read OD to know Ab concentrations.
11. OD (Absorbance at 280 nm)/1.35 = X mg/ml.
12. Store Abs at -20ºC or further concentrate by using Centriprep. And store at -20ºC.
13. Regenerate Ab column with Regenerate buffer (~200 ml).