LP109 – Protein A Purification of Antibodies

The following is a general protocol for Protein A purification of antibodies from serum. Recombinant Protein A agarose resin binds human IgG at approximately 25 mg/ml resin. Serum contains approximately 10 mg/ml of total IgG, tissue culture supernatants contain 20-50 µg of monoclonal antibody and ascites between 1 and 10 mg/ml.

Materials

Rainin Rabbit Plus Peristaltic pump
1.6mm ID Silicone tubing (Bio-rad #7318211)
2mm ID peristaltic tubing connector (Gilson F1825113)
Kontes Flex-Column 1.5 x 5cm (VWR 420400-1505)
Luer locks (Bio-rad #7318222, 7318225, 73128102)
5ml collection tubes and rack (Sarstedt 55.526)
Eppendorf BioPhotometer and Uvettes
Protein A Agarose beads (ABT PA09-R5)
Dialysis tubing and closures (Spectra Por 4 - 132700)
4L Beaker
Magnetic stirrer and stir bar
Filtration units (Nalgene 166-0045)

Solutions

1M Tris: Tris, pH8.0

100 mM Tris: 50 ml 1M Tris pH 8.0, fill to 500 ml with distilled water.

Binding Buffer: 50 mM Tris, pH8.0: 25 ml 1M Tris, pH8.0, fill to 500 ml with distilled water.

10 mM Tris: 5 ml 1M Tris pH8.0, fill to 500 ml with distilled water.

Elution buffer: 100 mM Glycine, pH2.5

Wash buffer: 50 mM Diethanolamine, pH11. Store at 4°C

1 x TBS: 50 mM Tris, 100 mM NaCl, pH7.4

1 x TBS/Azide: 1X TBS, 0.1% Na Azide

1 x PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4. pH7.4

Filter sterilize all buffers before use.

Protocol

1. Make a 5 ml bead-bed in the column, slowly pour beads into column and rinse bottle with distilled water and add to column. Tap to remove air bubbles. Leave an extra ml of liquid above bead bed, do not let beads dry out.
2. Set pump to 0.5-1 ml/min (Speed 4 = 1 ml/min. Will be different each time depending on size of column, tubing etc.)
3. Wash column with 25 ml (5 column volumes) of distilled water to eliminate the preservative
4. Equilibrate column with 25 ml (5 column volumes) of binding buffer, 50mM Tris, pH8.0.
5. Centrifuge serum at 4000 rpm for 10 min at 4 °C to eliminate aggregates and debris.
6. Dilute sample 1:1 in binding buffer and apply 20 ml (~100mg of IgG) to the column. Recirculate once then collect flow-through in 1 fraction.
7. Wash the column with 25 ml 100 mM Tris pH8.0 – collect in 1 fraction. Check OD280 periodically
8. Wash the column with 50 ml 10 mM Tris pH8.0 – collect in 1 fraction. Check OD280 again.
9. Elute the antibody with 20 ml 100 mM Glycine, pH2.5
10. Collect fractions of 2 ml in collection tubes containing 200 µl 1M Tris, pH8.0.
11. Mix each tube gently to neutralize, avoid foaming as this denatures the protein.
12. Check fractions after 20 ml. Identify the immunoglobulin containing fractions by measuring absorbance at 280 nm. Pool concentrated fractions together and pool other fractions separately.
13. Wash column with 1 bed-volume of 10 mM Tris pH8.0, collect fraction
14. Wash Protein A column with 20 ml 50 mM Diethanolamine pH11. Collect in 2 ml fractions and check by measuring absorbance at 280 nm.
15. Wash column with 4 column volumes (20 ml) of glycine buffer and then 20 ml of diethanolamine followed by 20 ml TBS and then 2 column volumes (10 ml) of TBS/Azide. Store column in TBS/Azide buffer at 4°C immediately after use.
16. Run all fractions on reducing and non-reducing gels to check purity, degradation etc.
17. Pool appropriate fractions together
18. Dialyze overnight in 100x volume PBS, repeat dialysis in fresh PBS.
19. Measure absorbance again at 280 nm and calculate concentration.
20. QC – Run 1µg on a reducing and non-reducing gel to check for degradation, concentration.
21. Test activity by ELISA if necessary.