

LP113 – Antigen-Affinity Purification of Antibodies

The following is a general protocol for affinity purification of antibodies from serum. Peptides or proteins are covalently bound to agarose beads by their free sulfhydryls, Pierce SulfoLink Coupling Resin can bind approximately 1 mg sulfhydryl containing peptide per 1 ml of settled resin. The peptide-agarose column is then used to purify the antibody from the serum. An affinity column can typically be re-used up to 10 times depending on the stability of the immobilized peptide.

Materials

Pierce SulfoLink Coupling Resin (20401)
Peptide or Protein
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
Dialysis tubing and closures (Spectra Por 4 - 132700)
4L Beaker
Magnetic stirrer and stir bar
Filtration units (Nalgene 166-0045)

Solutions

Coupling Buffer: 50mM Tris, 5mM EDTA-Na, pH8.5

Quenching Reagent: 50mM L-Cysteine HCl in Coupling Buffer, prepare fresh each time

Wash Solution: 1M Sodium Chloride

Storage Buffer: PBS + 0.05% Sodium Azide

Binding/Wash buffer: 50mM 1 x TBS

Elution buffer: 100mM Glycine, pH2.5

50mM Diethanolamine pH11. Store at 4°C

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

1 x TBS/Azide: 1 x TBS + 0.1% Azide

1 x PBS: 137mM NaCl, 2.7mM KCl, 10mM Na₂HPO₄, 2mM KH₂PO₄. pH7.4

Filter sterilize all buffers before use.

Protocol

Coupling Peptide to the Resin

1. Equilibrate Sulfolink Resin, peptide and reagents to room temperature.
2. Set pump to 0.5-1ml/min (Speed 4 = 1 ml/min. Will be different each time depending on size of column, tubing etc.)
3. Swirl resin to evenly suspend and then transfer 10ml to a column to give a 5ml bed volume
4. Equilibrate column with 20ml (4 column volumes) Coupling Buffer, do not allow resin bed to dry.
5. Resuspend 5mg peptide in 5ml Coupling Buffer, take OD280 reading.
6. Drain remaining buffer from column and add 5ml of peptide into top of column.
7. Replace cap and mix by inverting until beads are completely in suspension. Continue to mix by placing on a rocker for 15min at room temperature.
8. Stand the column upright and incubate at room temperature for 30min to allow beads to settle
9. Remove bottom cap and allow buffer to drain, keep flow through and take OD280 reading again to assess coupling efficiency.
10. Wash column with 15ml Coupling Buffer and allow to drain.
11. Replace bottom cap and add 5ml Quenching Reagent to top of column and repeat steps 7 and 8.
12. Wash the column with 50ml Wash Buffer (1M NaCl)
13. Wash with 15ml Storage Buffer
14. Column can now be stored upright at 4°C or proceed with affinity purification.

Affinity Purification of Antibody

1. Equilibrate column with 15ml (3 column volumes) of Binding/Wash buffer, TBS
2. Centrifuge serum at 4000rpm for 10min at 4 °C to eliminate aggregates and debris.
3. Dilute sample 1:1 in Binding/Wash buffer and apply 20ml (~100mg of IgG) to the column. Recirculate once then collect flow-through in 1 fraction.
4. Wash the column with 25ml TBS. Check OD280 periodically
5. Elute the antibody with 20ml 100mM Glycine, pH2.5
6. Collect fractions of 1ml in collection tubes containing 100µl 1M Tris, pH8.0, antibody will come off quickly in a small peak.
7. Mix each tube gently to neutralize, avoid foaming as this denatures the protein
8. Check fractions after 20ml. Identify the immunoglobulin containing fractions by measuring absorbance at 280nm. Pool concentrated fractions together and pool other fractions separately.



9. Wash and equilibrate column with 40ml Binding/Wash Buffer.
10. Between rabbits wash column with 20ml 50mM Diethanolamine pH11. Collect in 2ml fractions and check by measuring absorbance at 280nm.
11. Wash column with 4 column volumes (20ml) of glycine buffer and then 20ml of diethanolamine followed by 20ml TBS and then 2 column volumes (10ml) of TBS/Azide. Store column in TBS/Azide buffer at 4°C immediately after use.
12. Run all fractions on reducing and non-reducing gels to check purity, degradation etc.
13. Pool appropriate fractions together
14. Dialyze overnight in 100x volume PBS, repeat dialysis in fresh PBS.
15. Measure absorbance again at 280nm and calculate concentration.
16. QC – Run 1µg on a reducing and non-reducing gel to check for degradation, concentration.
17. Direct ELISA using peptide coated plate.