

Protocol

Preparation of Antibody Samples

Average IgM concentration in human serum is about 1.5 mg/mL. To ensure proper ionic strength and pH for optimal binding, mix ascites fluid, serum, or tissue culture supernatant sample with Binding Buffer at a ratio of 1:3. Centrifuge samples at 10,000 x g for 5 minutes to remove any insoluble material and use only the clear supernatant for antibody purification.

1. The resin can be used at 2-25 °C. Pour about 1 mL IgM affinity matrix into the column. Remove the bottom cap to allow the storage solution to drain through the column.
2. Slowly add 5-10 mL of Binding Buffer to the top of the resin. Allow the column to drain until liquid level drops to resin level.
3. Add a clarified, diluted antibody sample (5-10 mL) to the column and allow it to slowly pass through the immobilized ligand (Maximum column capacity per purification is about 6-12 mg IgM).
4. Add 10 mL of Binding Buffer and allow it to pass through the column to wash away nonbound components (or until you see a stable baseline).
5. Add 4 mL of Elution Buffer and collect 1 mL elution fractions. Immediately add 100 µL of Neutralization Buffer to each of the 1 mL elution fractions.
6. Measuring OD 280nm or Protein assay to identify and combine elution fractions that contain the purified antibody. (Optional) Alternatively, use a desalting column or dialysis to exchange the purified antibody into a more suitable buffer for long-term storage.
7. Regenerate the column by washing with 10 mL of Elution Buffer and followed by 5 mL of 20% Ethanol. When approximately 1 mL of solution remains above the resin, cap the column and store upright at 4°C. Columns may be regenerated a minimal of 9 times without significant loss of binding capacity.

Related Products:

Protein A Agarose (#C8054)
 Antibody Purification Kit (#C8056-A)
 ELISA Washing Buffer(20x, 25mL; #C8003)
 Protein Loading Buffer(6x ; #C8005)
 Phosphate Buffered Saline (10x PBS, #C8007)

Protein G Agarose (#C8055)
 Antibody Purification Kit (#C8056-G)
 Antibody Dilution Buffer(#C8004)
 SDS-PAGE Running Buffer(10x ; #C8006)
 Phosphate Buffered Saline with Tween 20(10x PBS-T; #C8008)