ab193260 – Protein G Sepharose® Column

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab193260

Storage and Stability

On receipt, store at 4°C. The beads may be damaged above 40°C. DO NOT FREEZE. Stable, as supplied, for at least 1 year.

Contents

Ready-to-use pre-packed columns of 1 ml or 5 ml bead volume in 20 % Ethanol/dH2O

Features

- High binding capacity: Binding of IgG >20 mg human or rabbit IgG/mL Protein G Sepharose®.
- Minimal leaching of the ligand
- Flow Rate Tested*: 2.07 mL/min.
  *Test condition: Calculations based on the time required to pass 18 mL of water through 2 mL settled beads (column diameter 1.5 cm).
- Usage: Reusable for up to 10 times without significant loss of binding capacity.

Wash beads 3 times with 3x bead volume of desired buffer before use.

Applications

- Purification of monoclonal and polyclonal antibodies from culture media, serum, ascites fluid or hybridoma supernatants.
- Isolation of antibody/antigen complexes in immunoprecipitation experiments, since only the Fc region is involved in antibody binding and the Fab region is available for binding antigen.

Protocol Example (Antibody Purification)

1. Carefully pack the column avoiding air bubbles.
2. Equilibrate the column with 5X resin bed volume of Binding Buffer & allow the buffer to drain through the column. Do not let the resin bed dry.
3. Dilute serum sample with Binding Buffer (1:1 ratio).
4. Mix well the diluted serum sample. Make sure there are no bubbles in the sample solution.
5. Apply the diluted sample onto the column. Do not let the resin bed dry.
6. Collect the flow-through.
7. Reapply the flow-through to the column & collect the sample. Repeat 4 times.
8. Wash the column 4 - 5 times with 5X volume of Binding Buffer containing an additional 0.5 M NaCl.
9. Wash the column 4 - 5 times with Binding Buffer.
10. Elute antibodies with Elution Buffer ~3-5X resin bed volume.
11. Collect fractions using micro centrifuge tube. Immediately neutralize the eluted fractions by adding 100 µl of 1 M Tris, pH 9.0 per ml of eluate.
12. Assay protein concentration by measuring the absorbance at 280 nm and combine the fractions with highest absorbance. 1 OD280 = 0.73 mg/ml IgG.
13. To regenerate/store column:
    a. Wash with 5 volumes of Elution Buffer.
    b. Wash with 5 volumes of distilled water.
    c. Store column in 20 % Ethanol/H_2O at 4°C.

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Buffers

Binding Buffer:

- PBS, or
- TBS, or
- 0.15 M sodium chloride in 50 mM sodium borate, pH 8.0

Elution Buffer:

- 0.1 M citric acid, pH 2.75

Sepharose is a registered trademark of GE Healthcare

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)