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ab193257
Protein A Sepharose® Column

For the purification of monoclonal and polyclonal antibodies.

View Protein A Sepharose® Column datasheet:
www.abcam.com/ab193257
[use www.abcam.cn/ab193257 for China, or www.abcam.co.jp/ab193257 for Japan)

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Protein A Sepharose® Column ab193257 display high chemical & physical stability as well as high flow rate, hydrophilicity & high gel strength. It can be used for IgG purification and immunoprecipitation.

The columns are prepared by covalently coupling recombinant Protein A to 6% cross-linked Sepharose beads. The coupling technique is optimized to give a higher binding capacity for IgG & minimum leaching of recombinant Protein A. The IgG binding capacity of Protein A-Sepharose Column is ≥ 16 mg human or rabbit IgG per mL of wet beads.

The columns are used for the purification of monoclonal and polyclonal antibodies from culture media, serum, ascites fluid or hybridoma supernatants. They are also used in the 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.

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.

2. Materials Supplied and Storage

Store at 4°C. Do not freeze. Stable, as supplied, for at least 1 year.

Ready-to-use pre-packed columns.

Item	Quantity	Storage temperature
Protein A/G/L Sepharose® Column	1 mL/5 mL	4°C

3. Materials Required but not supplied

Binding Buffers: PBS/TBS/0.15 M sodium chloride in 50 mM sodium borate, pH 8.0.

Elution Buffers: 0.1 M citric acid, pH 2.75.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Assay Procedure

1.1 Carefully pack the column avoiding air bubbles.
1.2 Equilibrate the column with 5X resin bed volume of Binding Buffer and allow the buffer to drain through the column. Do not let the resin bed dry.
1.3 Dilute serum sample with Binding Buffer (1:1 ratio).
1.4 Mix the diluted serum sample well. Make sure there are no bubbles in the sample solution.
1.5 Apply the diluted sample onto the column. Do not let the resin bed dry.
1.6 Collect the flow-through.
1.7 Reapply the flow-through to the column & collect the sample. Repeat 4 times.
1.8 Wash the column 4 – 5 times with 5X volume of Binding Buffer containing 0.5 M NaCl.
1.9 Wash the column 4 - 5 times with Binding Buffer.
1.10 Elute antibodies with Elution Buffer ~3-5X resin bed volume.
1.11 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.
1.12 Assay protein concentration by measuring the absorbance at 280 nm and combine the fractions with highest absorbance. 1 OD_{280} = 0.73 mg/mL IgG.
1.13 To regenerate/store column:

1.13.1 Wash with 5 volumes of Elution Buffer.
1.13.2 Wash with 5 volumes of distilled water.
1.13.3 Store column in 20 % Ethanol/H_2O at 4°C. Store upright at 4°C.

6. Notes

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