ab288103 – Protein A Antibody Purification Kit

A simple, ready to use kit containing all necessary buffers and pre-packed columns to purify up to 350 mg of IgG.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab288103

Storage and Stability

Store kit at 4°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
Hi-BindTM Protein Column	1 column	+4°C
Binding Buffer (10X)	40 ml	+4°C
Elution Buffer (10X)	20 ml	+4°C
Neutralization Buffer	10 ml	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 20% Ethanol
- 1.5 ml Collection Tubes
- 50 ml Centrifuge Tubes

Reagent Preparation

- Briefly centrifuge small vials prior to opening.
- Read the entire protocol before purifying IgG.

Binding Buffer: Dilute Binding Buffer (10X) with ddH2O. (i.e. add 1 ml Binding buffer (10X) to 9 ml ddH2O). Diluted buffer is stable at 4°C for 3 months.

Elution Buffer: Dilute Elution Buffer (10X) with ddH2O. (i.e. add 1 ml Elution buffer (10X) to 9 ml ddH2O). Diluted buffer is stable at 4°C for 3 months.

Antibody Purification Protocol

1. Centrifuge samples at 10000 x g at 4°C for 25 min and collect the supernatant to a new tube. Equilibrate samples by mixing 1:1 (v/v) of sample with Binding Buffer. The maximum loading volume is 4 ml at once. (For larger sample size, equilibrate samples by mixing 9 volume of sample with 1 volume of 10X Binding Buffer.)
2. Remove the top cap and bottom cap respectively. Allow the column to drain the storage buffer. Equilibrate the column by adding 5 ml Binding Buffer. Do not let resin bead dry out at any time.
3. Place a clean 50 ml Centrifuge Tube underneath the Column. Gradually load the equilibrated sample into the Column and let it flow through the Column. Reload the non-adsorbed material 8 to 10 times at RT to maximize the IgG binding capacity.
4. Collect the final flow through. Wash the Column twice with 5 ml Binding Buffer.

   ΔNote: *Keep the flow through until satisfactory enrichment of IgG in eluate is confirmed.*

5. Prepare 10 collection tubes (label 1-10) with 100 µl Neutralization Buffer in each tube. Put the Column in tube #1 and add 1 ml Elution Buffer in the Column. Mix the eluted samples with the Neutralization Buffer immediately. Repeat the elution step for another 6-9 times, each time with a new Collection Tube.

6. **Measurement:** Measure the IgG concentration for each fraction by measuring absorbance at 280 nm (1.4 OD280 ≈ 1 mg/ml IgG). Combine the eluted fractions containing purified IgG.

7. **Column Regeneration:** Wash the Column with 10 ml Elution Buffer, followed by 5 ml distilled water twice and then 5 ml Binding Buffer twice. Add 5 ml 20% ethanol into the Column, drain out ~3 ml, and store the Column at 4 °C. Column can be regenerated up to 10 times without significant loss of binding capacity. We recommend reusing the column only for the same sample to avoid cross contamination.

Technical Support

For all technical or commercial enquiries please go to:

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