ab288095 – Protein G Spin Antibody Purification Kit

For the fast and efficient IgG purification in serum, ascites and cell culture medium from various species.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab288095

Storage and Stability

- Store kit at 4°C in dark. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.
- All buffers are ready to use.

Materials Supplied

Item	Quantity	Storage Condition
Hi-Bind Protein G Spin-Column	10 columns	-4°C
Equilibration Buffer	1 mL	-4°C
Wash Buffer	28 mL	-4°C
Elution Buffer	10 mL	-4°C
Neutralization Buffer	2 x 1.3 mL	-4°C

Materials Required, Not Supplied

- Micro centrifuge tubes (1.5 ml)

Reagent Preparation and Storage Conditions

- Store kit at 4°C in dark.
- Briefly centrifuge small vials prior to opening.
- Read entire protocol before performing the assay.
- All buffers are ready to use.

Sample Preparation

Centrifuge samples at 10,000 x g and 4°C for 25 minutes and transfer supernatant to new tubes. Equilibrate samples by mixing with Equilibration Buffer at ratio of 10:1. (Ex. mix 90 µl of sample with 10 µl Equilibration Buffer). The maximum loading volume is 0.6 ml.
Note: IgG amount should be lower than 3 mg/column to avoid losses in flow through.

Protocol

1. Snap off the bottom plug from the spin column by twisting it gently and save for later use. Put a micro centrifuge tube at the bottom to collect flow-through.
2. Centrifuge the column at 700 x g for 2 min (use same conditions for all washes and eluates) to remove storage buffer. Discard flow-through.
3. Prepare Binding Buffer by 1:10 dilution of Sample Equilibration Buffer with H2Odi (i.e. 600µl to 6 ml). Wash and equilibrate the column twice with 0.25 ml Binding Buffer.
4. Put the snap back to the bottom of the column and load the equilibrated sample into it, plug the column with the top cap.
5. Incubate the column for 1 hour at room temperature or overnight at 4°C by slowly inverting the column to achieve mixing of sample and beads.
6. Unplug the cap and the bottom plug, insert the column in a clean micro centrifuge tube and spin the column at 700 x g for 2 min to collect non-adsorbed material.
7. Wash the column with 0.25 ml Wash Buffer and centrifuge at 700 x g for 2 min. Repeat this step three more times using new micro centrifuge tube every time. Monitor the absorbance of the washes at 280 nm (A280) and perform additional washes if necessary, until the absorbance approaches baseline.

Note: Keep the flow through and washes until satisfactory enrichment of IgG in eluate is confirmed.

8. Prepare 8 micro centrifuge tubes (label 1-8) with 20 µl Neutralization Buffer in each tube. Place the column inside tube #1 and add 0.1 ml Elution Buffer in the column. Incubate the column for 1-2 min then centrifuge at 700 x g for 2 min.
9. Mix the eluted solution with Neutralization Buffer immediately. Repeat elution step 3-5 times, each time collecting in a new micro centrifuge tube.

Analysis

Measure the IgG concentration by measuring OD absorbance at 280 nm. (1.4 OD280 = 1 mg/ml IgG) Combine the eluted fractions containing the purified IgG.

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)

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