

ab288107 – Protein A Spin Antibody Purification Kit

For the Purification of monoclonal and polyclonal antibodies from culture media, serum, ascites fluid or hybridoma supernatants.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<https://www.abcam.com/ab288107>

Storage and Stability

The kit and contents can be stored at 4°C in the dark.

Materials Supplied

Item	Quantity	Storage Condition
Hi-Bind Protein A Spin-Column	10 columns	4°C
Equilibration Buffer	1 ml	4°C
Binding Buffer	25 ml	4°C
Elution Buffer	10 ml	4°C
Neutralization Buffer	1 ml	4°C

Materials Required, Not Supplied

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

- 1.5 ml microcentrifuge tubes

Reagent Preparation

- Briefly centrifuge small vials prior to opening.
- All buffers are ready to use.
- Read entire protocol before performing the assay.

Sample Preparation

- Centrifuge samples at 10000 x g at 4°C for 25 minutes and transfer supernatant to new tubes. Equilibrate samples by mixing with Equilibration Buffer at ratio of 10:1. (e.g. mix 90 µl of sample with 10 µl Equilibration Buffer)

Δ Note: IgG amount should be lower than 3 mg/column.

Assay Protocol

1. Protein A Spin-Column Preparation: Snap off the bottom plug from the spin column by twisting it gently and save for later use. Position a microcentrifuge tube at the bottom of the column to collect flow-through.
2. Centrifuge the column at 700 x g for 2 min (use the same conditions for all washes and eluates) to remove storage buffer. Discard flow-through. Wash and equilibrate the column twice with 0.25 ml Binding Buffer.
3. Sample Incubation: Return the plug to the bottom of the column and load the equilibrated sample into it. Plug the column with the top cap. 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.
4. Washing: Unplug the cap and the bottom plug and spin the column at 700 x g for 2 min to collect non-adsorbed material. Wash the column with 0.25 ml Binding Buffer and centrifuge at 700 x g for 2 min. Repeat this step three more times. Monitor the absorbance of the washes at 280 nm (A280) and perform additional washes if necessary, until the absorbance approaches baseline.

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

5. Elution: Prepare 6 micro centrifuge tubes (label 1-6) with 10 µl Neutralization Buffer in each tube. Put 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. Mix the eluted solution with Neutralization Buffer immediately. Repeat elution step 3-5 times, each time collecting eluate in a new micro centrifuge tube.

Calculations

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

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