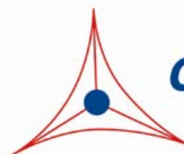
Product Manual

Rapid Antibody Purification Kit

Catalog Number

AKR- 160	10 preps

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



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Introduction

Cell Biolabs' Rapid Antibody Purification kit is designed for rapid, single-step purification of high-quality IgG from ascites, serum and tissue culture media or hybridoma supernatants. Samples containing IgG are incubated with immobilized Protein A in a buffer that facilitates binding. After non-IgG components are washed from the column, the bound IgG is recovered by elution. The supplied buffers provide for maximum immunoglobulin binding and elution efficiency with the Protein A column. The Rapid Antibody Purification Kit contains a Protein A agarose column suitable for a total of 10 purification preps; each prep can purify the IgG amounts specified in Table 1 below.

Species	mg of IgG per prep
Bovine	15-20
Goat	6-12
Human	20-30
Mouse	6-12
Rabbit	15-20

Table 1. Maximum column capacity per purification prep.

Related Products

1. AKR-102: Phospho Antibody Stripping Solution
2. AKR-103: PhosphoBLOCKER™ Blocking Reagent
3. AKR-150: Mouse Antibody Isotyping Kit
4. AKR-152: Mouse Antibody Isotyping Kit with Kappa & Lambda Light Chains

Kit Components

1. Protein A Agarose Column (Part No. 216001): One column with 1 mL Protein A resin
2. Binding Buffer (Part No. 216002): Two 200 mL bottles
3. 5X Elution Buffer (Part No. 216003): One 100 mL bottle
4. Neutralization Buffer (Part No. 216004): One 10 mL bottle

Storage

Store kit components at room temperature.

Preparation of Reagents

- 1X Elution Buffer: Dilute the 5X Elution Buffer Concentrate to 1X with deionized water. Stir to homogeneity.

Preparation of Antibody Samples

To ensure proper ionic strength and pH for optimal binding, mix ascites fluid, serum, or tissue culture supernatant sample with Binding Buffer at a ratio of 1:1. Centrifuge samples at 10,000 x g for 5 minutes to remove any insoluble material and use only the clear supernatant for antibody purification.

Protocol

Note: Air bubbles may become trapped inside the column which can significantly reduce flow rates. To remove these bubbles, cap the bottom of the column, add 2-3 mL of Binding Buffer to the top of the column, and replace the cap on the top of the column. Then either shake the column vigorously by hand for several seconds, or place the column in a 15 mL conical tube and centrifuge at 1000 g for 5 minutes.

1. Invert the Protein A Agarose Column several times to resuspend the agarose beads. Remove the top cap from the Protein A column first, then remove the bottom cap to allow the storage solution to drain through the column.
2. Slowly add 5 mL of Binding Buffer to the top of the resin. Allow the column to drain until liquid level drops to resin level.
3. Apply up to 5 mL of diluted antibody sample to the equilibrated Protein A column and allow antibody sample to flow through the column.

   Note: For optimal recovery, do not apply more than the maximum IgG per prep as shown in Table 1 above.
4. Wash column with 10 mL of Binding Buffer and allow it to drain through the column.
5. Elute the bound antibody with 5 mL of Elution Buffer and collect 1 mL elution fractions.
6. Immediately add 50 uL of Neutralization Buffer to each of the 1 mL elution fractions. Determine which fractions contain antibody by measuring the absorbance of each one at 280 nm or by protein assay. Pool fractions having the highest amount of proteins (typically fractions 2, 3 and 4).
7. (optional) Exchange the eluted antibody into desired storage buffer by dialysis or gel filtration.
8. Regenerate the column by washing with 10 mL of Elution Buffer and followed by 5 mL of water containing 0.02% sodium azide. When approximately 2 mL of solution remains above the resin, cap the column and store upright at 4ºC. Columns may be regenerated a minimum of 9 times without significant loss of binding capacity.

Recent Product Citations

1. Keshari, D. et al. (2017). MSMEG_5684 down-regulation in Mycobacterium smegmatis affects its permeability, survival under stress and persistence. *Tuberculosis*. **103**:61-70 doi: 10.1016/j.tube.2017.01.004.

2. Sharma, R. et al. (2016). MRA_1571 is required for isoleucine biosynthesis and improves Mycobacterium tuberculosis H37Ra survival under stress. *Sci Rep*. doi:10.1038/srep27997.
3. Ye, J. et al. (2015). Tissue-specific expression pattern and histological distribution of NLRP3 in Chinese yellow chicken. *Vet Res Commun.* **39**:171-177.

Warranty

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