Product Manual

Rapid Antibody Purification Kit

Catalog Number
AKR-160 10 preps

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures
**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.

<table>
<thead>
<tr>
<th>Species</th>
<th>mg of IgG per prep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>15-20</td>
</tr>
<tr>
<td>Goat</td>
<td>6-12</td>
</tr>
<tr>
<td>Human</td>
<td>20-30</td>
</tr>
<tr>
<td>Mouse</td>
<td>6-12</td>
</tr>
<tr>
<td>Rabbit</td>
<td>15-20</td>
</tr>
</tbody>
</table>

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 4°C. Columns may be regenerated a minimum of 9 times without significant loss of binding capacity.

**Recent Product Citations**


**Warranty**

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS’s sole obligation and purchaser’s exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

**Contact Information**

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

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