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Protein A Antibody Purification Kit

rev 02/20

(Catalog No. K974-10; 10 purifications; Store at 4°C)

I. Introduction:

Protein A beads have been widely used for IgG purification for their ability to bind immunoglobulins. BioVision's Protein A (Cat. No. 6500 and 6500B) is a genetically engineered protein containing five IgG-binding regions of native Protein A. Maximum IgG specificity is achieved by eliminating its cell wall binding region, albumin binding region, and other non-specific regions. Hi-Bind™ Protein A-Agarose beads (Cat. No. 6520) for purification of IgG contain covalently coupled recombinant Protein A to 6% cross-linked Agarose beads, the most popular resin for protein purification. **BioVision's Protein A Antibody purification Kit** is a simple, ready to use kit containing all necessary buffers and pre-packed columns to purify up to 350 mg of IgG. The kit can be used to purify antibodies in serum, ascites and cell culture medium from various species such as human, mouse, rat, goat and rabbit.

II. Application:

Antibody purification

III. Sample Types:

Serum, ascites and cell culture media

IV. Kit Contents:

Components	K974-10	Cap Code	Part Number
Hi-Bind™ Protein Column	1 column	--	K974-10-1
Binding Buffer (10X)	40 ml	NM	K974-10-2
Elution Buffer (10X)	20 ml	WM	K974-10-3
Neutralization Buffer	10 ml	NM	K974-10-4

V. User Supplied Reagents and Equipment:

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

VI. Specifications:

Species	IgG Binding Capacity (mg/ml)
Human	>30
Mouse	>30
Rat	>20
Goat	>15
Rabbit	>30

VII. Storage Condition and Reagent Preparation:

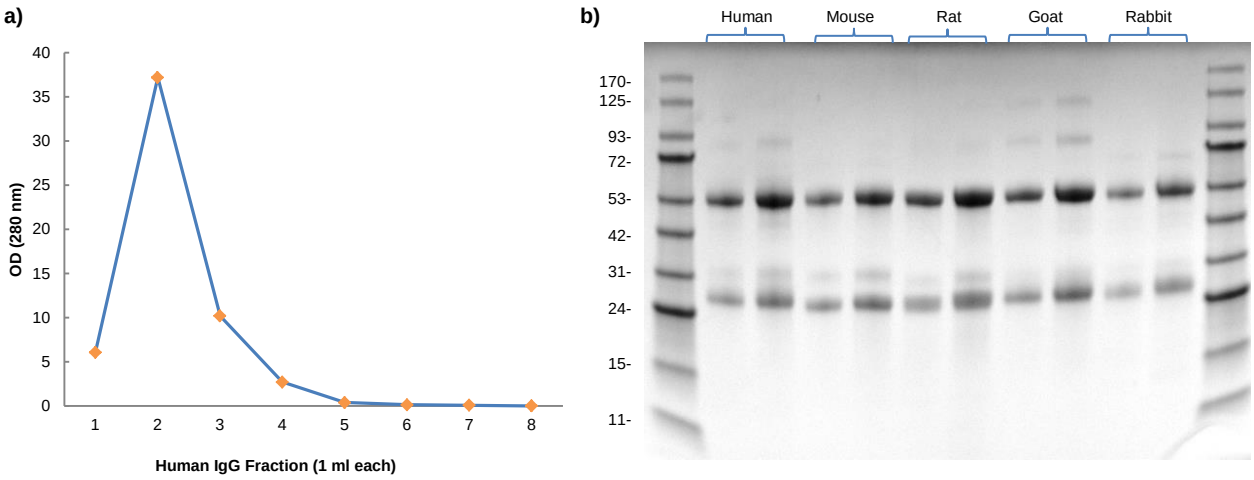
Store kit at 4°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before purifying IgG.

- **Binding Buffer:** Dilute Binding Buffer (10X) with ddH_2O. (i.e. add 1 ml Binding buffer (10X) to 9 ml ddH_2O). Diluted buffer is stable at 4°C for 3 months.
- **Elution Buffer:** Dilute Elution Buffer (10X) with ddH_2O. (i.e. add 1 ml Elution buffer (10X) to 9 ml ddH_2O). Diluted buffer is stable at 4°C for 3 months.

VIII. Antibody Purification Protocol:

1. **Sample Preparation:** 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. **Protein A Column Preparation:** 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. **Note: Do not let resin bead dry out anytime**
3. **Sample Loading:** 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. **Washing:** Collect the final flow through. Wash the Column twice with 5 ml Binding Buffer. **Note: Keep the flow through until satisfactory enrichment of IgG in eluate is confirmed.**
5. **Elution:** 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. **Analyses:** 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.

   Note: 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.



Figures: a) Human IgG elution curve: Protein A Antibody Purification Kit binding capacity was tested with 10 ml human serum. The sample was incubated overnight at 4°C. IgG amount as a function of order of eluted fractions **b)** SDS-PAGE of purified IgG from different species using Protein A Antibody Purification Kit (5 or 10 µg IgG per lane from 5 different species).

IX. RELATED PRODUCTS:

Hi-Bind™ Protein A-Agarose beads (6520)
Western Blot Substrate Kit (K820)
BCA Protein Quantitation Kit (K812, K813, K814)
Protease & Phosphatase inhibitor cocktails (K283, K284)
Protease inhibitor cocktails (K271, K272, K277, K278, K279)
Protein A (6500, 6500B)
Protein Quantitation kit (K810)
Protein Carbonyl Content Assay Kit (K830)
Membrane Protein Extraction Kit (K268)

FOR RESEARCH USE ONLY! Not to be used on humans.