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Protein A Spin Antibody Purification Kit

rev.03/19

(Catalog # K942-10; 10 columns; 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) 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 Mini Antibody Purification Kit is a simple, ready to use kit containing all necessary buffers and pre-packed columns to purify up to 30 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. Applications:

Antibody purification

III. Sample Type:

Serum, ascites and cell culture media

IV. Kit Contents:

Components	K942-10	Cap Code	Part Number
Hi-Bind™ Protein A Spin-Column	10 columns	--	K942-10-1
Equilibration Buffer	1 ml	Orange	K942-10-2
Binding Buffer	25 ml	WM	K942-10-3
Elution Buffer	10 ml	NM	K942-10-4
Neutralization Buffer	1 ml	Blue	K942-10-5

V. User Supplied Reagents and Equipment:

- Micro centrifuge tubes (1.5 ml)

VI. Specifications:

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

VII. Storage Conditions and Reagent Preparation:

- 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.

VIII. Antibody Purification Kit Protocol:

1. **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. (Ex. mix 90 µl of sample with 10 µl Equilibration Buffer)
   Note: IgG amount should be lower than 3 mg/column.
2. **Protein A Spin-Column Preparation**: 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. 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. Wash and equilibrate the column twice with 0.25 ml Binding Buffer.
3. **Sample Incubation**: Put the snap back 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.
   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 collection in a new micro centrifuge tube.
6. **Analyses**: Measure the IgG concentration by measuring OD absorbance at 280 nm. (1.4 OD_{280} = 1 mg/ml IgG) Combine the eluted fractions containing the purified IgG.

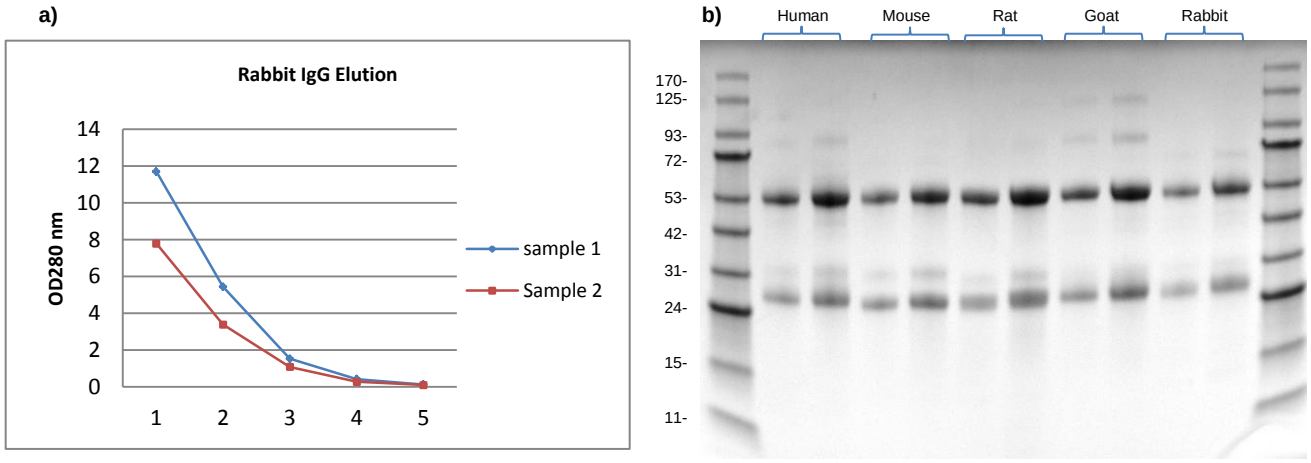

Figure: a) Rabbit IgG elution curve: Two rabbit serum samples were eluted using Protein A Spin Antibody Purification Kit. Each sample was incubated for 1 hour at room temperature. IgG amount as a function of order of eluted fractions. **b) SDS-PAGE**. SDS-PAGE of purified IgG from different species using Protein A Spin 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 (Cat# 6500, Cat#. 6520)
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.