



Protein G Spin Antibody Purification Kit

rev 02/20

(Catalog. No. K546-10; 10 columns; Store at 4°C)

• Introduction:

Protein G beads are widely used for IgG purification for their ability to bind selectively immunoglobulins. BioVision's Protein G (Cat. No. 6510) is a genetically engineered protein containing three Ig-binding regions of native Protein G. The cell wall binding region, albumin binding region and other non-specific regions have been eliminated from the recombinant Protein G to ensure the maximum specific IgG binding. The Hi-Bind™ Protein G Agarose beads display high chemical & physical stability as well as high flow rate, hydrophilicity & high gel strength. BioVision's Protein G Spin Antibody Purification Kit is a simple, ready to use kit containing all necessary buffers and pre-packed columns to purify up to 3 mg of IgG per sample in ten samples (30 mg IgG total). 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.

• Applications:

Antibody purification

• Sample Type:

Serum, ascites and cell culture media

• Kit Contents:

Components	K546-10	Cap Code	Part Number
Hi-Bind™ Protein G Spin-Column	10 columns	--	K546-10-1
Equilibration Buffer	1 ml	Orange	K546-10-2
Wash Buffer	28 ml	NM	K546-10-3
Elution Buffer	10 ml	NM	K546-10-4
Neutralization Buffer	1.3 ml X2	Blue	K546-10-5

• User Supplied Reagents and Equipment:

- Micro centrifuge tubes (1.5 ml)

• Specifications:

Species	IgG Binding Capacity (mg/ml)
Human	>30
Rabbit	>30

• 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.

• Antibody Purification Kit Protocol:

1. **Sample Preparation:** Centrifuge samples at 10,000 x g and 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). The maximum loading volume is 0.6 ml.

Note: IgG amount should be lower than 3 mg/column to avoid losses in flow through.

2. **Protein G 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. Prepare Binding Buffer by 1:10 dilution of Sample Equilibration Buffer with H₂Odi (i.e. 600µl to 6 ml). 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.

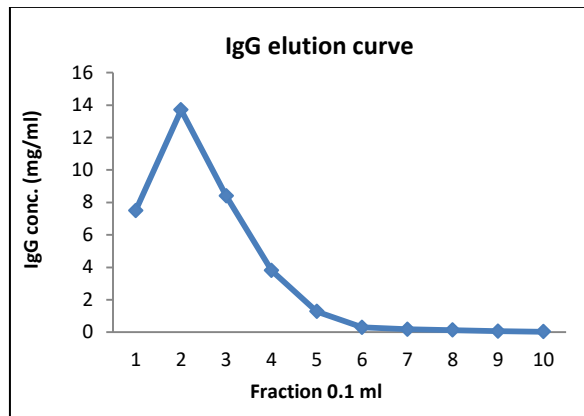
4. **Washing:** Unplug the cap and the bottom plug, insert the column in a clean micro centrifuge tube and spin the column at 700 x g for 2 min to collect non-adsorbed material. Wash the column with 0.25 ml Wash Buffer and centrifuge at 700 x g for 2 min. Repeat this step three more times using new micro centrifuge tube every time. 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 8 micro centrifuge tubes (label 1-8) with 20 µl Neutralization Buffer in each tube. Place 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 in a new micro centrifuge tube.

6. **Analyses:** 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.

a)



b)

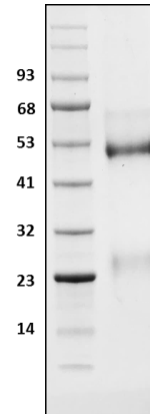


Figure: a) **Binding capacity:** Rabbit serum samples were eluted using Protein G Spin Antibody Purification Kit. Sample was incubated for 1 hour at room temperature. IgG amount as a function of order of eluted fractions. b) **SDS-PAGE:** SDS-PAGE of purified Rabbit IgG (2 µg) using Protein G Spin Antibody Purification Kit.

• **RELATED PRODUCTS:**

Hi-Bind™ Protein G-Agarose beads (6513)
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 G (Cat# 6510,)
Protein Quantitation kit (K810)
Protein Carbonyl Content Assay Kit (K830)
Membrane Protein Extraction Kit (K268)

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