

ToxOut™ Protein G (Sepharose) Spin Antibody Purification Kit 07/19

(Catalog # K2506-5 5 columns; Store at 4 °C)

I. 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. Protein G-Sepharose beads (Cat# M1301) for IgG purification contain covalently coupled recombinant Protein G to 6% cross-linked Sepharose beads, the most popular resin for protein purification. BioVision's ToxOut™ Protein G (Sepharose) Spin Antibody Purification Kit is a simple, ready to use kit for endotoxin-free IgG purification and immunoprecipitation. This kit can rapidly purify IgG from serum, ascites and cell culture media from various species such as human, mouse, rat, goat and rabbit.

II. Applications:

Antibody purification for endotoxin-free samples

III. Sample Types:

Serum, ascites and cell culture media

IV. Kit Contents:

Components	K2506-5	Part Number
ToxOut™ Protein G (Sepharose) Spin Column	5 columns	K2506-5-1
ToxOut™ Equilibration Buffer	1.8 ml	K2506-5-2
ToxOut™ Binding Buffer	10 ml x 2	K2506-5-3
ToxOut™ Elution Buffer	10 ml	K2506-5-4
ToxOut™ Neutralization Buffer	1.5 ml	K2506-5-5
ToxOut™ Collection Tube	50 tubes	K2506-5-6

V. Specifications:

ToxOut™ Protein A (Sepharose) Spin Column capacity is different in different species: ~2 mg/column

Species	IgG Binding Strength
Human	++++
Mouse	++++
Rat	+
Goat	+
Rabbit	++++

Note: Strong Binding +++++/ Weak Binding+

VI. User Supplied Reagents and Equipment:

- Endotoxin-free 15 ml centrifuge tubes

VII. Storage and Handling:

- Store at 4°C. All buffers expire 2 months after opening.
- Read the entire protocol before performing the assay.
- Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.
- To prevent endotoxin contamination from dust, solution or dirty lab ware, only use endotoxin-free solutions and tubes and proceed with extra caution.
- Do not let the resin dry out anytime.

VIII. Antibody Purification Kit Protocol:

- 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 9:1. (ex. mix 90 µl of sample with 10 µl Equilibration Buffer)

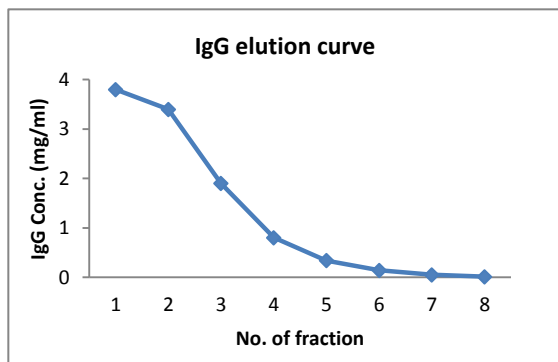
Note: Total IgG should be < 2 mg/column to ensure elution rate.

- Protein G Spin-Column Preparation:** Snap off the bottom plug from spin column by twisting it gently and save for later use. Put a ToxOut™ Collection Tube at the bottom to collect flow-through. Centrifuge the column at 700 x g for 2 min (use this step for all washes and eluates) to remove storage buffer. Discard flow-through. Wash and equilibrate the column 2 more times with 0.3 ml Binding Buffer each time.
- Sample Incubation:** Put the bottom plug back, load the equilibrated sample and plug the top cap back. Incubate the column for 1 hour at room temperature or overnight at 4°C by slowly inverting the column to achieve maximum binding.
- Washing:** Unplug both top cap and bottom plug and spin the column at 700 x g for 2 min to collect non-adsorbed material. Wash the column with 0.3 ml Binding Buffer and centrifuge at 700 x g for 2 min. Repeat this step 4 times.

Note: Reserve all flow through and washes in a 15 ml centrifuge tube until satisfactory enrichment of IgG elution is confirmed.

5. **Elution:** Prepare 6 ToxOut™ collection tubes (labeled 1-6) by adding 20 μ l Neutralization Buffer in each tube. Put the column inside tube #1 and add 0.1 ml Elution Buffer. Incubate the column for 1-2 min then centrifuge at 700 x g for 2 min. Mix the eluate with the Neutralization Buffer inside the tube immediately. Repeat elution step 3-5 times, each time in a new ToxOut™ collection tube.
6. **Analyses:** Measure the IgG concentration by measuring OD absorbance at 280 nm. ($1.4 \text{ OD}_{280} = 1 \text{ mg/ml IgG}$) Combine the eluted fractions containing the purified IgG.

a)



b)

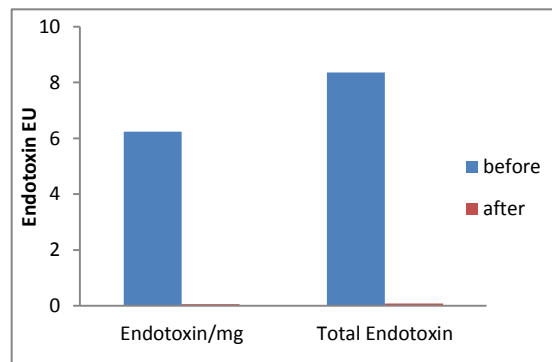


Figure: a) Binding capacity test: IgG Elution curve using rabbit serum sample. Sample was incubated for 1 hour at room temperature and eluted with 0.1 ml Elution Buffer in each fraction. The IgG recovery rate is 90%. **b) ToxOut™ Protein G Sepharose endotoxin test:** Endotoxin level in concentration (right) and total amount (left) before and after purification of rabbit IgG with ToxOut™ Protein G (Sepharose) Spin Antibody Purification Kit. The recovered IgG eluate has > 98% reduction of endotoxin.

IX. RELATED PRODUCTS:

- ToxOut™ Rapid Endotoxin Removal Kit (K2501)
- ToxOut™ Protein A (Sepharose) Antibody Purification Kit (K2503)
- ToxOut™ Protein A (Sepharose) Column (M2503)
- ToxOut™ Endotoxin-Free Protein A Sepharose (M1300)
- ToxOut™ Endotoxin-Free Protein G Sepharose (M1301)
- Protein G (Cat# 6510)

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