



# ToxOut™ Protein A (Sephacrose) Spin Antibody Purification Kit 12/18

(Catalog # K2504-5, 5 columns; Store at 4 °C)

## I. Introduction:

Protein A beads have been widely used for IgG purification. 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. ToxOut™ Endotoxin-free Protein A-Sepharose beads (Cat.# M1300) for IgG purification contain covalently coupled recombinant Protein A to 6% cross-linked sepharose beads, the most popular resin for protein purification. BioVision's ToxOut™ Protein A (Sephacrose) 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	K2504-5	Part Number
ToxOut™ Protein A (Sephacrose) Spin Column	5 columns	K2504-5-1
ToxOut™ Equilibration Buffer	1.8 ml	K2504-5-2
ToxOut™ Binding Buffer	10 ml X2	K2504-5-3
ToxOut™ Elution Buffer	10 ml	K2504-5-4
ToxOut™ Neutralization Buffer	1.5 ml	K2504-5-5
ToxOut™ Collection Tube	50 tubes	K2504-5-6

## V. Specifications:

ToxOut™ Protein A (Sephacrose) 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:

1. **Sample Preparation:** Centrifuge samples at 10,000 x g at 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.

2. **Protein A 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.

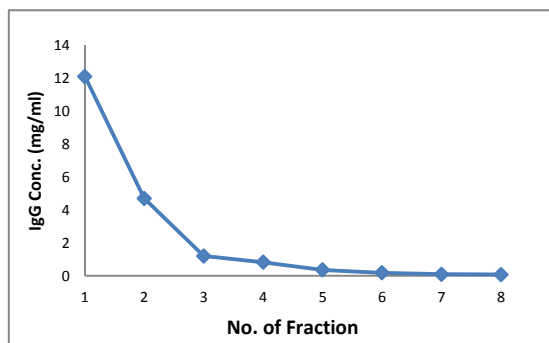
3. **Sample Incubation:** Put the bottom plug back, load the equilibrated sample and plug the top cap back. Incubate the column for 1 hour at room temperature or overnight at 4°C by slowly inverting the column to achieve maximum binding.

4. **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 more times.

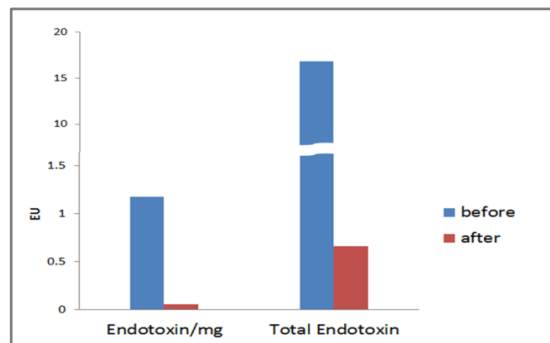
**Note:** Reserve all flow through and washes in a 15-ml centrifuge tube until satisfactory enrichment of IgG elution is confirmed.

5. **Elution:** Prepare 6 ToxOut™ collection tubes (label 1-6) with 20 µl 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 eluted solution with Neutralization Buffer 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. b) **ToxOut™ Endotoxin-Free Protein A Sepharose endotoxin test:** Endotoxin level in concentration (left) and total amount (right) before and after rabbit IgG were purified. The recovered IgG shows > 96% endotoxin reduction.

#### 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 A (Cat# 6500, 6520)

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