



ToxOut™ Protein A (Sephacel) Antibody Purification Kit

(Catalog# K2503-1, 1 column, Store at 4°C)

12/18

I. Introduction:

Protein A beads have been widely used for IgG purification. BioVision's Protein A (Cat# 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 (Cat#M1300) for IgG purification contain covalently coupled recombinant Protein A to 6% cross-linked sepharose beads, the most popular resin for protein purification. ToxOut™ Protein A (Sephacel) Antibody Purification Kit is a 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. Application:

Antibody purification for endotoxin-free samples

III. Kit Contents:

Components	K2503-1	Part Number
ToxOut™ Protein A (Sephacel) Column	1 column	K2503-1-1
ToxOut™ Equilibration Buffer	1.8 ml	K2503-1-2
ToxOut™ Binding Buffer	10 ml X 3	K2503-1-3
ToxOut™ Elution Buffer	10 ml	K2503-1-4
ToxOut™ Neutralization Buffer	1.5 ml	K2503-1-5
ToxOut™ Collection Tube	10 tubes	K2503-1-6

IV. User Supplied Reagents and Equipment:

- Endotoxin-Free 50-ml centrifuge tubes

V. Storage and Handling:

- Store at 4°C. All buffers expire 2 months after opening.
- Read the 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.

VI. Specification:

ToxOut™ Protein A (Sephacel) Column capacity: ~20mg

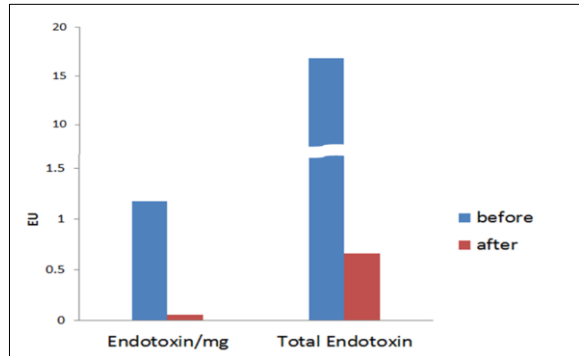
Species	IgG Binding Strength
Human	++++
Mouse	++++
Rabbit	++++
Rat	+
Goat	+

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

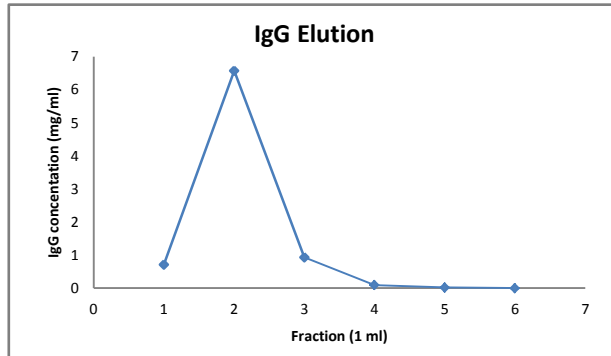
VII. Antibody Purification Protocol:

- Sample Preparation:** Centrifuge samples at 10,000 xg at 4 °C for 25 min to remove insoluble materials. If sample volume is < 5 ml, equilibrate supernatant with ToxOut™ Binding Buffer at 1:1 (v/v). If sample volume is > 5 ml, mix supernatant with ToxOut™ Equilibration Buffer at ratio of 9:1 (v/v) in a new tube. Concentrate the sample if it is in large volume (> 10ml).
- Column Preparation:** Remove the top cap and then the bottom cap. Drain the storage buffer from the column. Equilibrate the column by adding 5 ml ToxOut™ Binding Buffer.
- Sample Loading:** Place a clean endotoxin-free 50-ml tube underneath the column. Load equilibrated sample and allow it slowly pass through the column. Repeat this step 8-10 times for maximum IgG binding.
- Washing:** Drain out the sample and wash the column twice with 5 ml ToxOut™ Binding Buffer each. (Note: Keep the flow through and first 2 ml washing until satisfactory enrichment of IgG in eluate is confirmed.)
- Elution:** Prepare 10 collection tubes (label 1-10) with 150 µl ToxOut™ Neutralization Buffer in each tube. Put the column on tube #1 and add 1 ml ToxOut™ Elution Buffer in the column. Mix the eluted samples with neutralization buffer immediately. Repeat elution step for another 6-9 times, each time with a new collection tube.
- Collection:** Measure IgG concentration for each fraction by OD280nm (1.4 OD280 ≈ 1 mg/ml IgG) and combine the elution fractions which contain purified IgG.

a)



b)



Figures a) Endotoxin level in concentration (left) and total amount (right) before and after rabbit IgG were purified by ToxOut™ Protein A (Sepharose) Antibody Purification Kit. The recovered IgG shows > 96% endotoxin reduction. **b)** Elution curve for rabbit IgG purification using this kit. The IgG recovery rate from first three elution fraction is 91.5%.

VIII. Related Products:

- ToxOut™ Rapid Endotoxin Removal Kit (K2501)
- ToxOut™ High Capacity Endotoxin Removal Kit (K2502)
- ToxOut™ Protein A (Sepharose) Spin Antibody Purification Kit (K2504)
- ToxOut™ Protein A (Sepharose) Column (M2503)
- ToxOut™ Endotoxin-Free Protein A Sepharose (M1300)
- ToxOut™ Endotoxin-Free Protein G Sepharose (M1301)
- ToxOut™ Endotoxin Free Collection Tube (7937)
- Protein A (Cat# 6500, 6520)