*FOR RESEARCH USE ONLY!*

# ToxOut™ Endotoxin-Free Protein A Sepharose

*rev10/18*

**Store at 4°C. Do not freeze.**

**Cat. No.**

**M1300-1 Protein A-Sepharose, 1 ml settled resin**
**M1300-5 Protein A-Sepharose, 5 ml settled resin**
**M1300-25 Protein A-Sepharose, 25 ml settled resin**

**Support:** 6% cross-linked Sepharose beads supplied as 50% slurry (e.g., 1 ml of settled resin is equivalent to 2 ml of 50% slurry) in 20% Ethanol/$H_2O$.

**Binding Capacity:** >15 mg human or rabbit IgG/ml of settled resin.

**Flow Rate Tested*:** 0.85 cm/min.
*Test condition: Linear flow rate determined in 2 ml column with internal diameter of 1.5 cm.

**Introduction:**
Protein A is a cell wall component produced by several strains of *staphylococcus aureus*. This bacteria-derived protein binds with high affinity & specificity to the Fc portion of antibodies, especially with IgG class. Therefore, Protein A has been widely used for IgG purification. BioVision's Protein A (Cat. No. 6500, Cat. No. 6500B) is a genetically engineered protein containing five IgG-binding regions of native Protein A. The cell wall binding region, albumin binding region and other non-specific regions have been eliminated from the recombinant Protein A to ensure the maximum specific IgG binding. Protein A-Sepharose beads display high chemical & physical stability as well as high flow rate, hydrophilicity & high gel strength. It can be used for IgG purification and immunoprecipitation. Protein A-Sepharose beads are prepared by covalently coupling recombinant Protein A to 6% cross-linked Sepharose beads. The coupling technique is optimized to give a higher binding capacity for IgG & minimum leaching of recombinant Protein A. The IgG binding capacity of Protein A-Sepharose is ≥ 15 mg human or rabbit IgG per ml of wet beads. Endotoxin-free Protein G-Sepharose is made under our proprietary endotoxin-free conditions. Our Endotoxin-free Protein A-Sepharose also shows ability of reducing/removing certain amount of endotoxin from serum or ascites samples.

**Applications:**
- Purification of endotoxin-free monoclonal and polyclonal antibodies from culture media, serum, ascites fluid or hybridoma supernatants.
- Isolation of antibody/antigen complexes in immunoprecipitation experiments, since only the Fc region is involved in antibody binding and the Fab region is available for binding antigen.

**User Supplied Reagents or Equipment (Endotoxin-Free reagents and equipment should be used in all procedures)**
- Binding Buffer: PBS/TBS/0.15 M sodium chloride in 50 mM sodium borate, pH 8.0
- Elution Buffer: 0.1 M citric acid, pH 2.75
- Neutralization Buffer: 1 M Tris-HCl, pH-9

**Protocol example (Antibody Purification):**

1. Carefully pack the column avoiding air bubbles.
2. Equilibrate the column with 5 resin volume of Binding Buffer & allow the buffer to drain through the column. Do not let the resin dry.
3. Dilute serum sample with Binding Buffer (1:1 ratio).
4. Mix well the diluted serum sample. Make sure there are no bubbles in the sample solution.
5. Apply the diluted sample onto the column. Do not let the resin dry.
6. Collect the flow-through.
7. Reapply the flow-through to the column & collect the sample. Repeat 4 times.
8. Wash the column 4 - 5 times with 5 volume of Binding Buffer containing 0.5 M NaCl.
9. Wash the column 4 - 5 times with Binding Buffer.
10. Elute antibodies with Elution Buffer ~3-5 resin volume. Collect fractions using micro centrifuge tube containing neutralization buffer (100 µl of 1 M Tris, pH 9.0 per ml of eluate).
11. Assay protein concentration by measuring the absorbance at 280 nm and combine the fractions with highest absorbance. 1 $OD_{280}$ = 0.73 mg/ml IgG.
12. To regenerate/store column:
    a. Wash with 5 volumes of Elution Buffer.
    b. Wash with 5 volumes of distilled water.
    c. Store column in 20 % Ethanol/$H_2O$ at 4°C.

    **Note:** Columns may be regenerated 8-10 times without significant loss of binding capacity.

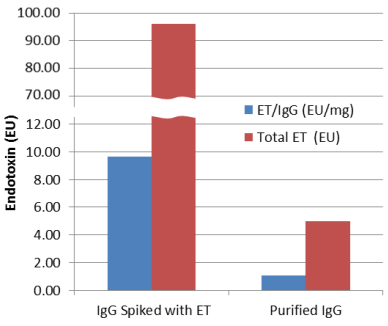


**Figure: IgG purification with protein A-Sepharose (Endotoxin-Free):** IgG (10 mg) spiked with endotoxin (96 EU) is loaded onto 1 ml Endotoxin-free protein A-Sepharose. After purification procedures, the recovered IgG shows even reduced endotoxin level (More than 90% reduction).

**APPENDIX: Protein A affinity for immunoglobulins**

| Species | Ig | Binding Strength |
|---|---|---|
| Human | Total IgG | ++++ |
| Human | IgG1 | ++++ |
| Human | IgG2 | ++++ |
| Human | IgG3 | + |
| Human | IgG4 | ++++ |
| Mouse | Total IgG | ++++ |
| Mouse | IgG1 | + |
| Mouse | IgG2a | ++++ |
| Mouse | IgG2b | ++++ |
| Mouse | IgG3 | ++++ |
| Rat | Total IgG | + |
| Rat | IgG1 | + |
| Rat | IgG2a | - |
| Rat | IgG2b | - |
| Rat | IgG2c | ++++ |
| Rabbit | Total IgG | ++++ |
| Pig | Total IgG | ++++ |
| Horse | Total IgG | + |
| Hamster | IgG | + |
| Guinea Pig | Total IgG | ++++ |
| Cow | Total IgG | + |
| Chicken | Total IgG | - |
| Goat | Total IgG | + |
| Dog | Total IgG | ++++ |
| Cat | Total IgG | ++++ |
| Sheep | Total IgG | + |

**Legend:** ++++: Strong Binding
++: Medium Binding
+: Weak Binding
-: No Binding

**RELATED PRODUCTS:**

- Hi-Bind™ Protein A-Agarose **(Cat. No. 6520)**
- Protein A-Agarose **(Cat. No. 6526)**
- Protein A-Sepharose **(Cat. No. 6501)**
- Protein A-Sepharose Column **(Cat. No. 6508)**
- Protein A-Magnetic Beads **(Cat. No. 6507)**
- Protein A Antibody **(Cat. No. 5500)**
- Protein A **(Cat. No. 6500, 6500B)**
- Protein A IgG Binding Buffer **(Cat. No. 6524)**
- IgG Elution Buffer **(Cat. No. 6525)**
- Protein A IgG Purification Buffer Kit **(Cat. No. 6529)**
- Hi-Bind™ Protein G-Agarose **(Cat. No. 6513)**
- Protein G-Sepharose **(Cat. No. 6511)**
- Protein G-Sepharose Column **(Cat. No. 6518)**
- Protein G-Magnetic Beads **(Cat. No. 6517)**
- Protein G **(Cat. No. 6510)**
- Protein G Antibody **(Cat. No. 5510)**
- Protein G-Biotin **(Cat. No. 6215)**
- Protein L-Sepharose **(Cat. No. 6531)**
- Protein L-Sepharose Column **(Cat. No. 6538)**
- Protein L Magnetic Beads **(Cat. No. 6537)**
- Protein L Antibody **(Cat. No. 5530)**
- Protein L **(Cat. No. 6530)**
- Protein A/G-Sepharose **(Cat. No. 6503)**
- Protein A/G-Sepharose Column **(Cat. No. 6528)**
- Protein A/G Magnetic Beads **(Cat. No. 6527)**
- Protein A/G **(Cat. No. 6502)**
- Protein A/G/L-Sepharose **(Cat. No. 6541)**
- Protein A/G/L-Sepharose Column **(Cat. No. 6548)**
- Protein A/G/L Magnetic Beads **(Cat. No. 6547)**
- Protein A/G/L **(Cat. No. 6540)**
- Protein G Coated 96-well Plate **(Cat. No. 6522)**

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