

# ToxOut™ Endotoxin-Free Protein A Agarose

rev 11/17

Store at 4°C. Do not freeze.

**Cat. No.****M1320-1 Protein A-Agarose, 1 ml settled resin****M1320-5 Protein A-Agarose, 5 ml settled resin****M1320-25 Protein A-Agarose, 25 ml settled resin**

**Support:** 6% cross-linked Agarose beads supplied as 50% slurry (e.g., 1 ml of settled resin is equivalent to 2 ml of 50% slurry) in 20% Ethanol/H<sub>2</sub>O.

**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-Agarose 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-Agarose beads are prepared by covalently coupling recombinant Protein A to 6% cross-linked Agarose 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-Agarose is ≥ 15 mg human or rabbit IgG per ml of settled beads. Our Endotoxin-free Protein A-Agarose also shows ability of reducing/removing certain amount of endotoxin from serum or ascites samples.

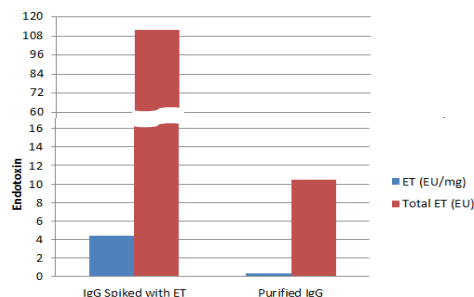
**Applications:**

- 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.

**Protocol example (Antibody Purification):**

1. Carefully pack the column avoiding air bubbles.
2. Equilibrate the column with 5 resin volume of endotoxin-free Binding Buffer (i.e. PBS) & allow the buffer to drain through the column. Do not let the resin dry.
3. Dilute serum sample with endotoxin-free 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 endotoxin-free Binding Buffer containing 0.5 M NaCl.
9. Wash the column 4 - 5 times with endotoxin-free Binding Buffer.
10. Elute antibodies with endotoxin-free Elution Buffer (i.e. 0.1 M Citric Acid) ~3-5 resin volume. Collect fractions using micro centrifuge tube containing endotoxin-free 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<sub>280</sub> = 0.73 mg/ml IgG.

**Figure: IgG purification with protein A-Agarose (Endotoxin-Free).** IgG (11.73 mg) spiked with endotoxin (111.98 EU) is loaded onto 1 ml Endotoxin-free protein A-Agarose. After purification, the recovered IgG shows significantly reduced endotoxin levels (<1EU/mg).



**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:**

- Protein A Sepharose (Endotoxin-free) (Cat. No. **M1300**)
- Protein G Sepharose (Endotoxin-free) (Cat. No. **M1301**)
- 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**)
- Hi-Bind™ Protein A-Agarose (Cat. No. **6520**)
- 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**)

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