

Protein A-Sepharose Column

rev. 11/12

Catalog #	6508-1	1 ml
	6508-5	5 ml

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. # 6500, Cat. # 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 Columns display high chemical & physical stability as well as high flow rate, hydrophilicity & high gel strength. It can be used for IgG purification and immunoprecipitation.

PREPARATION:

Protein A-Sepharose Columns 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 Column is ≥ 16 mg human or rabbit IgG per ml of wet beads.

APPLICATIONS:

- Purification of 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.

CONTENTS: Ready-to-use pre-packed columns of 1 ml or 5 ml bead volume in 20 % Ethanol/dH₂O.

STORAGE: Store at 4°C. Do not freeze. Stable, as supplied, for at least 1 year.

BINDING CAPACITY: Binding of IgG ≥ 16 mg human or rabbit IgG/ml Protein A-Sepharose.

FLOW RATE TESTED*: 2.07 ml/min

*Test condition: Calculations based on the time required to pass 18 ml of water through 2 ml settled beads (column diameter 1.5 cm).

USAGE: Reusable for up to 10 times without significant loss of binding capacity.

PROTOCOL EXAMPLE (ANTIBODY PURIFICATION):

1. Carefully pack the column avoiding air bubbles.
2. Equilibrate the column with 5X resin bed volume of Binding Buffer & allow the buffer to drain through the column. Do not let the resin bed 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 bed 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 5X 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 ~3-5X resin bed volume.
11. Collect fractions using micro centrifuge tube. Immediately neutralize the eluted fractions by adding 100 μ l of 1 M Tris, pH 9.0 per ml of eluate.
12. Assay protein concentration by measuring the absorbance at 280 nm and combine the fractions with highest absorbance. 1 OD₂₈₀ = 0.73 mg/ml IgG.
13. 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/H₂O at 4°C.

BUFFERS:

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

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:

- Recombinant Protein A & Agarose & Magnetic Beads
- Recombinant Protein G & Agarose, Sepharose & Magnetic Beads
- Recombinant Protein L & Sepharose & Magnetic Beads
- Recombinant Protein A/G & Sepharose & Magnetic Beads
- Recombinant Protein A/G/L & Sepharose & Magnetic Beads
- Protein A Polyclonal Antibody
- Protein G Polyclonal Antibody
- Protein L Polyclonal Antibody

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