

Hi-Bind™ Protein A-Agarose

rev. 09/16

Store at 4°C, Do not freeze

Cat. No.

6520-1	Hi-Bind™ Protein A-Agarose,	1 ml settled resin
6520-5	Hi-Bind™ Protein A-Agarose,	5 ml settled resin
6520-25	Hi-Bind™ Protein A-Agarose,	25 ml settled resin
6520-100	Hi-Bind™ Protein A-Agarose,	100 ml settled resin
6520-500	Hi-Bind™ Protein A-Agarose,	500 ml settled resin
6520-1000	Hi-Bind™ Protein A-Agarose,	1 L settled resin

Support: 6% cross-linked Agarose beads supplied as 50% slurry in 20% Ethanol/H₂O (e.g., 1 ml of settled resin is equivalent to 2 ml of 50% slurry) in 20% Ethanol/H₂O

Binding Capacity: ≥ 36 mg human or rabbit IgG/ml Protein A-Agarose

***Flow Rate Tested:** 4 cm/min.

* 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. # 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. The Hi-Bind™ 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.

Preparation:

Hi-Bind™ Protein A-Agarose beads are specially prepared for high IgG binding by covalently coupling recombinant Protein A to 6% cross-linked Agarose beads, the most popular resin for protein affinity purification methods. The coupling technique is optimized to give a higher binding capacity for IgG & minimum leaching of recombinant Protein A than the other regular Protein-A Agarose on the market.

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.

User Supplied Reagents or Equipment:

- 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: 1.5 M Tris-HCl pH-8.8

For maximum binding & elution efficiency, we recommend using BioVision's Protein A IgG Purification Buffer Kit (Cat. # K6529-3) containing Protein A IgG Binding Buffer, IgG Elution Buffer & Neutralization Buffer. Protein A IgG Binding Buffer (Cat. # K6524), IgG Elution Buffer (Cat. # K6525) & Neutralization Buffer (Cat. # 1105) can also be purchased separately.

Protocol example (Antibody Purification):

- Sample preparation:** Centrifuge samples at 5000 x g for 15 min. at 4°C. Dilute supernatant at least 1:1 with Protein A IgG Binding Buffer. Make sure the ionic strength and pH are maintained for optimal binding.
- Column:** Equilibrate the Buffers & column to room temperature. Carefully pack the column avoiding air bubbles. Equilibrate the column with 5X resin bed volume of Protein A IgG Binding Buffer & allow the Buffer to drain through the column. Do not let the resin bed dry.
- Loading:** Add diluted samples to equilibrated column and allow it to flow completely into the resin.
Note: For max. yield, reapply the flow-through to the column & collect sample. Repeat 1-5 times.
- Washing:** Wash the column with at least 5-10 resin-bed volumes of Protein A IgG Binding Buffer.
- Elution:** Elute IgG with IgG Elution buffer and collect fractions in micro centrifuge tubes containing neutralization buffer (150 µl of 1.5 M Tris-HCl (pH-8.8) per ml of eluate). Measure the protein concentration by measuring absorbance at 280 nm and combine the fractions with highest absorbance. 1 OD₂₈₀ = 0.73 mg/ml IgG. Use the purified antibodies directly for WB, SDS-PAGE or dialyze for specific application. Purified IgG should be stored at -20°C.
- To regenerate/store column:** Reusable for up to 8-10 times without significant loss of binding capacity.
 - Wash with 5 volumes of Elution Buffer.
 - Wash with 5 volumes of distilled water.
 - Store column in 20 % Ethanol/H₂O at 4°C.

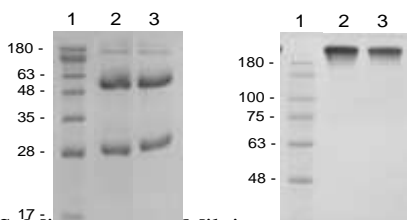


Figure: SDS-PAGE of purified IgG under reduced (a) and non-reduced conditions (b). Lane 1: Marker; Lane 2 & Lane 3: IgG fraction (5 µg) purified using BioVision Hi-Bind™ Protein A-Agarose (Cat # 6520) & Hi-Bind™ Protein G-Agarose (Cat # 6513) respectively.

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 (6520)
 Protein A-Sepharose (6501)
 Protein A-Magnetic Beads (6507)
 Protein A (6500, 6500B)
 IgG Elution Buffer (6525)
 Hi-Bind™ Protein G-Agarose (6513)
 Protein G-Sepharose Column (6518)
 Protein G (6510)
 Protein G Coated 96-well Plate (6522)
 Protein L-Sepharose (6531)
 Protein L Magnetic Beads (6537)
 Protein L (6530)
 Protein A/G-Sepharose Column (6528)
 Protein A/G (6502)
 Protein A/G/L-Sepharose Column (6548)
 Protein A/G/L (6540)

Protein A-Agarose (6526)
 Protein A-Sepharose Column (6508)
 Protein A Antibody (5500)
 Protein A IgG Binding Buffer (6524)
 Protein A IgG Purification Buffer Kit (6529)
 Protein G-Sepharose (6511)
 Protein G-Magnetic Beads (6517)
 Protein G Antibody (5510)
 Protein G-Biotin
 Protein L-Sepharose Column (6538)
 Protein L Antibody (5530)
 Protein A/G-Sepharose (6503)
 Protein A/G Magnetic Beads (6527)
 Protein A/G/L-Sepharose (6541)
 Protein A/G/L Magnetic Beads (6547)

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