FOR RESEARCH USE ONLY!

Protein A/G-Agarose

03/21

Catalog #	6525-1	1 ml
	6525-5	5 ml
	6525-25	25 ml
	6525-100	100 ml

INTRODUCTION:

Protein A/G-Agarose is a genetically engineered protein that combines the IgG binding profiles of both Protein A and Protein G. It is a gene fusion product. Protein A/G binds to all IgG subclasses from various mammalian species, including all IgGs that bind to both Protein A and Protein G, making it the ideal choice for purification of all kinds of polyclonal or monoclonal IgG antibodies. Protein A/G-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:

Protein A/G-Agarose beads are prepared by covalently coupling recombinant Protein A/G to 6% cross-linked Agarose beads, the most popular resin for Protein A/G affinity purification methods. The coupling technique is optimized to give a higher binding capacity for IgG & minimum leaching of recombinant Protein A/G. The IgG binding capacity of Protein A/G-Agarose is ≥ 20 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.

CONTENTS: Supplied as 50% slurry in 20% Ethanol/H_2O.

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

BINDING CAPACITY: ≥ 20 mg human or rabbit IgG/ml of Protein A/G-Agarose.

FLOW RATE TESTED*: 2.89 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).

MAXIMUM FLOW RATE$^+$ = 1800 cm/hr; minimum leaching of recombinant Protein A/G.
$^+$The highest flow that beads withstand for 1 min, without collapsing and the pressure reaching 1 MPa.

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. **Note:** Do not let the resin bed dry.
3. Dilute the serum sample with Binding Buffer (1:1 ratio).
4. Mix the diluted serum sample. **Note:** 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 the antibody with Elution Buffer ~3-5X resin bed volume.
11. Collect the fractions using micro centrifuge tube. Immediately neutralize the eluted fractions by adding 100 µl of 1 M Tris, pH 9.0 per ml of eluate.
12. Assay the protein concentration by measuring the absorbance at 280 nm and combine the fractions with highest absorbance. 1 $OD_{280\ nm}$= 0.73 mg/ml IgG.
13. To regenerate or store the column:
    a. Wash with 5 volumes of Elution Buffer.

b. Wash with 5 volumes of distilled water.
c. Store the column in 20 % Ethanol/H_2O at 4 °C.

BUFFERS:

Binding Buffer: PBS/TBS/0.15 M NaCl in 50 mM sodium borate, pH 8.0
Elution Buffer: 0.1 M citric acid, pH 2.75

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

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