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A Geno Technology, Inc. (USA) brand name

Immobilized Protein A/G

1ml Column Kit

(Cat. # 786-839)

INTRODUCTION

Immobilized Protein A/G consists of recombinant protein A/G ligand covalently immobilized onto 6% highly cross-linked agarose. The dynamic binding capacity will vary depending on several factors such as target antibody, flow rate etc.

Protein A/G binds well to IgG subclasses but does not bind IgA, IgM or serum albumin. This makes Protein A/G an excellent tool for purification and detection of monoclonal antibodies from IgG subclasses, without interference from IgA, IgM and serum albumin. Individual subclasses of monoclonals are likely to have a stronger affinity to the chimeric Protein A/G than to either Protein A or Protein G.

The Immobilized Protein A/G 1ml spin column kit is ideal for the small scale affinity purification of antibodies form a variety of samples. Each 1ml column enables purification of 1-13mg IgG from 0.5-2ml of serum or other sample.

ITEM(S) SUPPLIED

Part. #	Description	Size
442P	IgG Elution Buffer	2 x 100ml
001J	JAW Phosphate Buffered Saline Pack	For 1L
049I-C	Immobilized Protein A/G Resin*	5 x 1ml columns

**Immobilized Protein A/G is supplied as a 50% slurry in 20% ethanol/PBS solution*

STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store it refrigerated at 4°C, DO NOT FREEZE. This product is stable for 1 year at 4°C.

SPECIFICATIONS

- High binding capacity: 38mg human IgG/ml resin; >20mg sheep IgG/ml resin
- Ligand: Recombinant Streptococcal protein A/G lacking the albumin binding sites expressed in E. coli
- Bead size: 50-165µm
- Bead Structure: 6% highly cross-linked agarose

Species	Antibody Class	Protein A	Protein G	Protein A/G
Mouse	Total IgG	++++	++++	++++
	IgG_1	+	+++	+++
	IgG_{2a}	++++	++++	++++
	IgG_{2b}	++++	++++	++++
	IgG_3	+++	+++	++++
Human	Total IgG	++++	++++	++++
	IgG_1	++++	++++	++++
	IgG_2	++++	++++	++++
	IgG_3	+	++++	++++
	IgG_4	++++	++++	++++
Rat	Total IgG	+	++	+++
	IgG_1	-	+	+++
	IgG_{2a}	-	++++	++++
	IgG_{2b}	-	++	+
	IgG_{2c}	++	+++	++++
Hamster	Total IgG	++	++	++
Guinea Pig	Total IgG	++++	++	++++
Rabbit	Total IgG	++++	+++	++++
Horse	Total IgG	++	++++	++++
Cow	Total IgG	++	++++	++++
Pig	Total IgG	+++	++	++++
Sheep	Total IgG	+	++	++++
Goat	Total IgG	+	++	++++
Chicken	Total IgG	-	-	-

Table 1: Relative affinity of Protein A, Protein G & Protein A/G for Immunoglobulins

ADDITIONAL ITEMS REQUIRED

- Storage Buffer: 10mM NaH_2PO_4, 150mM NaCl, 2.7mM KCl, pH 7.4, 20% ethanol
- Neutralization Buffer: 1M Tris·HCl, pH8.5
- Centrifuge
- 15ml collection tubes

PREPARATION BEFORE USE

Binding Buffer: Add the entire contents of the JAW Phosphate Buffered Saline pack to 1 liter of deionized water. Stir until completely dissolved.

PROTOCOL: SPIN PURIFICATION

NOTE: *The Immobilized Protein A/G columns can be used up to 10 times without significant loss of binding capacity.*

1. Allow the buffers and columns to equilibrate to room temperature.
2. Prepare the sample by diluting to 2ml with Binding Buffer. This is the maximum volume for the column.
3. Place the columns in a 15ml collection tube and briefly centrifuge at 1,000xg for 1 minute to pack the resin.
4. Remove the rubber stopper from the bottom of the column and remove the top cap. Return the columns to the 15ml collection tube and centrifuge at 1,000xg for 1 minute to remove the storage buffer. Discard the flow through.
5. Equilibrate the resin by adding 2ml Binding Buffer to the column in a collection tube. Centrifuge the column for 1 minute at 1,000xg and discard the flow through. Repeat this step once.
6. Cap the bottom of the column and apply sample to the column by adding to the top of the resin. Cap the column.
7. Incubate the column for 10 minutes at room temperature with end-over-end mixing.
8. Remove the top cap and then the bottom cap. Place the spin column in a new 15ml collection tube and centrifuge at 1,000xg for 1 minute. The flow through contains the non-bound sample and can be analyzed to determine binding efficiency.
9. Transfer the column to a new collection tube and apply 2ml Binding Buffer to the resin. Centrifuge for 1 minute at 1,000xg. Repeat this wash step two additional times.
10. Add 100µl Neutralization Buffer to three clean 15ml collection tubes. Place the spin column in one of the tubes.
11. Add 1ml IgG Elution Buffer to the spin column, mix gently and then centrifuge for 1 minute at 1,000xg. Transfer the spin column to another collection tube containing Neutralization Buffer. Repeat this step two more times.
12. Identify the immunoglobulin-containing fractions by measuring absorbances at 280nm or using a suitable protein assay. (NI-Protein Assay Cat. # 786-005)

13. If using for further downstream applications, exchange the buffer with our SpinOUT™ desalting columns or Tube-O-DIALYZER™ dialysis devices.
14. To regenerate the column, wash the column twice with 3ml Elution Buffer, followed by two washes with 3ml Storage Buffer. Do not allow the resin to dry out.

PROTOCOL: GRAVITY FLOW PURIFICATION

NOTE: *The Immobilized Protein A/G columns can be used up to 10 times without significant loss of binding capacity.*

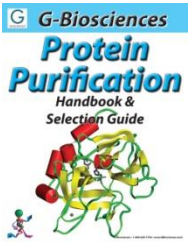
1. Allow the buffers and columns to equilibrate to room temperature.
2. Prepare the sample by diluting 1:1 with Binding Buffer.
3. Place the columns in a 15ml collection tube and briefly centrifuge at 1,000xg for 1 minute to pack the resin. If a suitable centrifuge is not available, simply tap the column to ensure all resin is at the bottom of the column.
4. Remove the rubber stopper from the bottom of the column and remove the top cap. Return the columns to the 15ml collection tube and allow the storage buffer to drain out. Discard the flow through.
5. Equilibrate the resin by adding 5ml Binding Buffer to the column and allow solution to drain out..
6. Apply the diluted sample to the column and collect the flow through.
   NOTE: *For optimal results, add a sample volume that is <80% the column's antibody binding capacity.*
   NOTE: *If excess antibody is present, then the flow through will contain this excess antibody. The flow through can be reapplied to a fresh or regenerated column later.*
7. Wash the column with 15ml Binding Buffer.
8. Add 100µl Neutralization Buffer to five clean 15ml collection tubes. Place the column in one of the tubes.
9. Add 1ml IgG Elution Buffer to the column and allow the elution to drain into the tube with Neutralization Buffer. Transfer the column to another collection tube containing Neutralization Buffer. Repeat this step a total of five times.
10. Identify the immunoglobulin-containing fractions by measuring absorbances at 280nm or using a suitable protein assay. (NI-Protein Assay Cat. # 786-005)
11. If using for further downstream applications, exchange the buffer with our SpinOUT™ desalting columns or Tube-O-DIALYZER™ dialysis devices.
12. To regenerate the column, wash the column with 8ml Elution Buffer, followed by 5ml Storage Buffer. Cap column when approximately 3ml Storage Buffer remains in the column. Do not allow the resin to dry out.

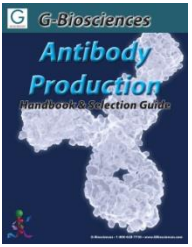
TROUBLESHOOTING

Issue	Possible Reason	Solution
No protein detected in eluted fractions	Initial sample is devoid of any antibody species or isotype that bind protein A/G.	Check Table 1 for relative affinity. Ensure by ELISA or isotyping that the sample contains the correct IgG type.
Large amount of antibody purified, but specific antibody of interest not detected	Antibody of interest has low affinity for the resin or is at low concentration	Use serum free media for cell supernatant samples
		Use affinity purification using a specific antigen coupled to a support (see Protein Purification Handbook for selection of supports)
Antibody of interest, but is denatured and inactive	Antibody is sensitive to low pH	Elute in a high salt buffer (5M LiCl, 10mM phosphate, pH7.2)
	Downstream application is sensitive to neutralized Elution Buffer	Desalt or dialyze sample

RELATED PRODUCTS

Download our Protein Purification and Antibody Production Handbooks.





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