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A Geno Technology, Inc. (USA) brand name

Immobilized Protein A/G

(Cat. # 786-836, 786-837, 786-838, 786-840)

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.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-836	Immobilized Protein A/G Resin*	3ml resin
786-837	Immobilized Protein A/G Resin*	15ml resin
786-838	Immobilized Protein A/G Resin*	5 x 1ml columns
786-840	Immobilized Protein A/G Resin*	10 x 0.2ml 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

- 1.0M Tris, pH 8.0
- 100mM Tris, pH 8.0
- 10mM Tris, pH 8.0
- 100mM Glycine, pH 3.0
- Storage Buffer: 10mM NaH_2PO_4, 150mM NaCl, 2.7mM KCl, pH 7.4, 20% ethanol
- Disposable columns

PREPARATION BEFORE USE

Sample preparation: We recommend that for optimal binding the serum samples/ascites fluid or tissue culture media be the addition of $1/10^{th}$ volume of 1.0M Tris, pH 8.0.

PROTOCOL

If using pre-packed columns(Cat. # 786-832, 786-834), start a step 2.

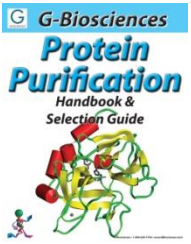
1. Add an appropriate volume of Protein A/G resin to a suitable disposable column. The table below is a guideline for an appropriate amount of resin for each ml of sample.

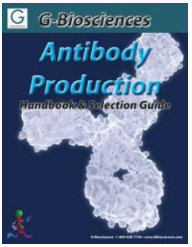
IgG Source	Bed volume (ml) / ml sample
Antisera	2ml
Tissue culture supernatant with 10% FBS	0.2ml
Tissue culture supernatant serum free	0.01ml
Ascites fluid	2ml

2. Equilibration Step: Wash the resin, by the addition of 10 column volumes (CV) of 100mM Tris, pH 8.0. Allow wash/binding buffers to drain under gravity.
3. Gently apply the sample to the column by adding to the top of the resin. Do not disturb the gel bed.
4. Wash Step: Wash the column with 10CV of 100mM Tris, pH 8.0 followed by 10CV 10mM Tris, pH 8.0 or until the absorbance (280nm) of the flow through is near or at background levels.
5. Elution Step: Elute the immunoglobulins from the column by adding 100mM Glycine, pH 3.0 in a stepwise manner. Add approximately 500µl per step up to a total volume of 4CV. Collect the eluate in 1.5ml tubes containing 50µl 1M Tris, pH 8.0.
6. Identify the immunoglobulin-containing fractions using a suitable protein assay. (NI-Protein Assay Cat. # 786-005)
7. Following elution, wash the resin with 2CV elution buffer, followed by at least 10CV 100mM Tris, pH 8.0. Store resin in storage buffer at 4°C.

RELATED PRODUCTS

Download our Protein Purification and Antibody Production Handbooks.





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Last saved: 9/24/2014 CMH

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