



Immobilized Protein A Resin

INTRODUCTION

Immobilized Protein A consists of a recombinant protein, covalently immobilized onto agarose. The recombinant protein A, produced in *E. coli*, has been designed to retain solely its binding properties for immunoglobulins. Immobilized protein A is widely used for the isolation and purification of a wide variety of immunoglobulins from a variety of species (see table below).

ITEMS SUPPLIED Cat. # 786-283

DESCRIPTION	Size
Protein A Resin*	5ml resin

*Immobilized Protein A Resin is supplied 10ml as 50% slurry in thimerosal/ H₂O solution

STORAGE CONDITIONS

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

SPECIFICATIONS

Binding Capacity: >20mg human IgG/ml resin

Bead Structure: 6% highly cross-linked agarose

SPECIES	ANTIBODY CLASS	PROTEIN A	PROTEIN 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 and Protein G for Immunoglobulins



PURIFICATION OF IgG MOLECULES

ITEMS NEEDED BUT NOT SUPPLIED WITH KIT

1.0M Tris, pH 8.0
100mM Tris, pH 8.0
10mM Tris, pH 8.0
100mM glycine, pH 3.0
Storage Buffer: 0.01M NaH₂PO₄, 0.15M 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 1/10th volume of 1.0M Tris, pH 8.0.

PROTOCOL

1) Add an appropriate volume of Protein A 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 buffer 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

1. Immobilized Protein G (Cat. # 786-284): For the binding and purification of IgG molecules. Different affinity compared to Protein A.

2. HOOK™ Agarose Coupling Kit (Sulphydryl reactive) (Cat.# 786-064): For the coupling of peptides and proteins to agarose through their sulphydryl groups.

Note: For other related products, please visit our web site at www.GBiosciences.com

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