Protein G Sepharose Pre-Packed Column

Catalog # 9077

For Research Use Only - Not Human or Therapeutic Use

DESCRIPTION:	Protein G: 4% cross-linked Sepharose
APPLICATION:	1. Purification of IgG from serum, ascites, and tissue culture media (1) 2. Purification of monoclonal IgG antibodies (1) 3. Isolation of immune complexes of IgG antibody-ligands 4. Immunoprecipitation with IgG antibodies against a ligand
QUANTITY:	1 ml (resin volume)
FORM:	Suspension in 20% ethanol (50% slurry)
BINDING CAPACITY:	Protein G: ≥20 mg (human IgG)/ml resin
STORAGE:	4 degrees C (DO NOT FREEZE)
STABILITY:	1 year
COLUMN DIMENSIONS:	9.5 cm x 1-2 cm
NOTES:	1. Affinity of Protein A/G for immunoglobulin may vary depending on the species, subclass, and subtype (Table 1). 2. Prolonged immersion of the resin in the elution solution can result in Protein A/G denaturation and affect the resin's future binding capacity.
REFERENCES:	1. S. M. Andrew, J. A. Titus, Purification of immunoglobulin G. *Curr. Protoc. Immunol. Chapter 2,* Unit 2.7 (2001).

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INTRODUCTION

Protein A and Protein G exhibit specific binding affinities for various immunoglobulin (Ig) subtypes, subclasses, and species, which are determined by the characteristics of the Ig Fc region. The details of their binding affinities are listed in Table 1 which categorizes the interaction strengths of Protein A and G with immunoglobulin subclasses across different species. Chondrex, Inc. offers ready-to-use prepacked protein A/G Sepharose columns which can be used for separating and purifying IgG antibodies from serum, ascites, and tissue culture media.

Table 1. Affinity of Protein A/G for Immunoglobulin Types from Different Species

Species	Immunoglobulin	Binding to Protein A	Binding to Protein G
Human	IgG (normal)	++++	++++
	IgG1	++++	++++
	IgG2	++++	++++
	IgG3	-	++++
	IgG4	++++	++++
	IgM	-	-
	IgA	-	-
	IgE	-	-
Mouse	IgG1	+	++++
	IgG2a	++++	++++
	IgG2b	+++	+++
	IgG3	++	+++
Rat	IgG1	-	+
	IgG2a	-	++++
	IgG2b	-	++
	IgG2c	+	++
Goat	IgG	+/-	++
Rabbit	IgG	++++	+++
Sheep	IgG	+/-	++

"-" : No binding, "+" : Weak binding, "++" : Binding, "+++" : Moderate Binding, "++++" : Strong Binding

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SAMPLE PROTOCOL (IgG Antibody Purification)

Buffer Preparation:

- Equilibration Buffer: 0.1M Tris-HCl, 0.15M NaCl, pH 7.5
- Elution Buffer: 0.1M Glycine-HCl, pH 2.7
- Neutralization buffer: 3.0 M Tris-HCl pH 8.0 buffer

Sample Preparation:

1. Dilute samples with an equal volume of the equilibration buffer.
2. Centrifuge at 10,000 rpm for 10 minutes
3. Collect the sample supernatant

Purification Protocol:

1. Ensure the resin in the column is packed at the bottom of the column.
2. Remove the cap, attach a 3-way stopcock, open the valve, and drain the storage solution in the column.
3. Equilibrate the column with 5 ml of equilibration buffer.
4. When equilibrating is complete, drain the column until no buffer remains in the resin and close the stopcock valve.
5. Load the sample supernatant onto the column and open the stopcock valve.
6. Pool the flow through sample.
7. Drain the column until no sample remains in the resin and close the stopcock valve.
8. Open the stopcock valve and wash the column with equilibration buffer.
9. Collect 5 to 15 tubes of 1 ml fractions. Keep collecting until the A280 OD value stabilizes.
10. Drain the column until no buffer remains in the resin and close the stopcock valve.
11. Add 100 µl of neutralization buffer into each elution collection tube.
12. Open the stopcock valve and elute the column with elution buffer.
13. Collect 5 to 15 tubes of 1 ml elution fractions and vortex each tube immediately to ensure the neutralization buffer mixes with the sample.
14. Check the A280 OD values of the eluted fractions.
15. Combine all positive fractions and dialyze the final sample with the dialysis buffer of choice.

    NOTE: the dialysis buffer will vary, depending on the type of sample and study. A commonly used one is 0.05M phosphate buffered saline, pH 7.4 (PBS).

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16. Open the stopcock valve and wash the column with 5 ml of distilled water.

17. Drain the column until no water remains in the resin and close the stopcock valve.

18. Open the stopcock valve and wash with 5 ml of distilled water containing 20% ethanol until the liquid level is several millimeters above the top of the resin bed (do not allow the resin to dry out).

19. Close the stopcock, replace the cap, and ensure all connections are tight and sealed.

20. Store at 4 degrees C.

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