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Product Information

Protein A-Immobilized

Product Number	Product Name	Binding Capacity (mg/ml)[d]
P1052	Protein A-Acrylic Beads (250 µm)[c]	Approx. 10
P8036	Protein A-Acrylic Beads (150 µm)[c]	Approx. 5
P0932	Protein A-Agarose CL-4B[b]	30-40
P7786	Protein A-Agarose[b]	Approx. 10
P2545	Protein A-Agarose CL-4B[a]	20-30
PA1	Prepacked Columns of P2545	20-25
P1406	Protein A-Agarose Cross-Linked[a]	20-30
P9269	Protein A-Agarose[a]	20-30
P1925	Protein A-Agarose 6MB[a]	Approx. 6
P0558	Protein A-Agarose 6MB[b]	10-20
P6649	Protein A-Sepharose® 6MB[a]	Approx. 6
P3391	Protein A-Sepharose CL-4B[a]	Approx. 20
P9424	Protein A-Sepharose 4B Fast Flow[a]	Approx. 35
P5906	Protein A-Agarose (Extracellular)[a]	20-30
P2670	Protein A-Agarose (Extracellular) Suspension[a]	20-30
54838	HiTrap® Protein A Column, 1 mL[e]	
54839	HiTrap Protein A Column, 5 mL[e]	
Z290068	SigmaChrom Pre-Packed HPLC Column, Protein A	Approx. 10

[a] Cyanogen Bromide Activated
[b] *p*-Nitrophenyl Chloroformate Activated
[c] Oxirane Activated
[d] Binding capacity determined using Human IgG (I4506).
[e] Separate data sheet is available. Please call Research Technical Service to request a copy.

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Buffers

Buffer A: 0.02 M NaH_2PO_4 (S0751) 2.4 g
0.15 M NaCl (S9625) 8.8 g
Adjust volume to 1 liter with water.
Adjust pH to 8.0.

Buffer B: 0.2 M Na_2HPO_4 (S0876) 25.7 mL
0.1 M Citric Acid (C7129) 24.3 mL
Water 50.0 mL
pH is dependent on species/subclass; see Table 1.

Swelling the Resin
Lyophilized products should be swollen in Buffer A for 30 minutes or longer at room temperature. Do not stir with any kind of mechanical stirrer. One gram of powder typically swells to 3-4 mL of hydrated gel. Resins can often be reused at least 5 times if stored and handled properly.

Storage/Stability
Store lyophilized powders at –20 °C. Store suspensions and hydrated resins at 2–8 °C in Buffer A with either 0.1% sodium azide, 20% ethanol, or 1% toluene as preservative.

DO NOT FREEZE LIQUIDS!

Procedure
Please refer to reference (1) for a review of Protein A binding to immunoglobulins (including extensive tables). The paper also covers immunoglobulin levels in sera. The use of Antibody-Sepharose in immunoprecipitation studies is described in reference (8); "Antibody-Sepharose" may be replaced with "Protein A-Sepharose."

Note: Tyrosine residues in the Fc region of IgG are involved with Protein A interactions. Glycyltyrosine may be used for elution (0.1 M glycyltyrosine in 2% NaCl, pH 7.0, at room temperature).[9,10]

Column Method
Make a 1:1 suspension of resin in Buffer A. Pour into column. Allow column to flow as it is settling. After it has settled, wash with 20 column volumes (CV) of Buffer A. Apply sample. Wash with 10 CV of Buffer A. Elute with 3 CV of Buffer B. Collect fractions. Neutralize the eluate with 0.1 M NaOH. Assay the eluate for IgG. Re-equilibrate the column with 20-30 CV of Buffer A. Store in Buffer A with a preservative at 2–8 °C. If solution volume is significantly greater than the resin volume, column method is recommended.

Batch Method
Equilibrate resin on a sintered glass funnel or Buchner funnel (with Whatman® 54 filter paper) by washing with 10 resin volumes (RV) of Buffer A using gentle vacuum. Combine resin and sample solution in a container. Gently mix suspension on a shaker for 1 hour (longer if the solution volume is significantly greater than the resin volume).

Collect the resin on the sintered flask or Buchner funnel. Wash with 10 RV of Buffer A. Transfer the resin to a beaker. Add twice the RV of Buffer B. Gently mix on shaker for 15 minutes. Collect resin on funnel as before, using a clean sidearm flask to collect the eluted antibody. Bring the eluate to neutral pH with 0.1 M NaOH. Wash the resin with 20 RV of Buffer A. Add preservative and store at 2–8 °C.

Cleaning Procedure
A decrease in the binding capacity may be due to steric hindrance by non-specifically bound proteins. It may be possible to clean the resin by washing it with 10-20 volumes of 100 mM Tris or borate buffer, pH 8.5, containing 0.5-2.0 M NaCl, followed by 10-20 volumes of 100 mM acetate buffer, pH 4.0, containing 0.5-2.0 M NaCl. Re-equilibrate the resin with 20 volumes of Buffer A. Add preservative and store at 2–8 °C.

Table 1.
Immunoglobin Binding

SPECIES	SUBCLASS	BINDING CAPACITY	ELUTION pH
Human	IgG	High	4
	IgG1	High	3.9-4.6
	IgG2	High	4.3-5
	IgG3	----	
	IgG4	High	3.9-5
Mouse	IgG1	Low[f]	6-7
	IgG2a	High	4.5-5
	IgG2b	High	4.5
	IgG3	High	3.5-4
Rabbit	IgG	High	3
Rat	IgG1	Low[f]	7
	IgG2a	----	
	IgG2b	----	
	IgG2c	Medium-High	3-4
Guinea Pig	IgG	High	4
Bovine	IgG	Low	
Goat	IgG	----[f]	

[f] Capacity may be increased by using alternative buffers: 1 M glycine with 2 M NaCl, pH 9 or 1 M borate with 2 M NaCl, pH 9. With mouse IgG1, use a higher pH (9), and a sodium chloride concentration of 2-3 M. Elute with a gradient to pH 3, 0.15 M NaCl.

References

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