

Protein A Agarose

Item No.
223-50-01

Size
5 mL



DESCRIPTION

Protein A Agarose consists of native protein A immobilized onto 4% cross linked agarose beads. It is designed specifically for the binding of immunoglobulins for both laboratory and process scale applications. The protein A molecule is very heat stable and retains its native conformation even after exposure to denaturing reagents such as 4M urea, 4M guanidine thiocyanate or 6M guanidine hydrochloride⁽¹⁾. Protein A binds specifically to the Fc region of immunoglobulin molecules of many mammalian species without disturbing their binding of antigen.

Covalently coupled Protein A Agarose has been extensively used for the isolation of a wide variety of immunoglobulin molecules from several mammalian species. Table 1 describes the relative affinity of immobilized Protein A for different antibody species and subclasses.

FORM/STORAGE

Protein A Agarose is supplied in a volume of 7 mL consisting of 5 mL Protein A agarose suspended in 20% ethanol/PBS. Store refrigerated at 2 - 8°C. Stable for a minimum of 1 year from date of receipt when stored at 2 - 8°C. Non-sterile.

SPECIFICATIONS

Ligand density:	~ 6mg Protein A/mL gel
Bead structure:	4% cross-linked agarose
Bead size range:	45 - 165 um
Recommended working pH:	3 - 9
Binding capacity:	>35mg/mL Human IgG

Note: Different immunoglobulins derived from the same species and from the same subclass can demonstrate deviations in the binding capacity.

Table 1. Relative Affinity of Immobilized Protein A for Various Antibody Species and Subclasses of polyclonal and monoclonal IgG's⁽²⁾.

<u>Species/ Subclass</u>	<u>Protein A</u>
MONOCLONAL	
Human	
IgG ₁	++++
IgG ₂	++++
IgG ₃	---
IgG ₄	++++
Mouse	
IgG ₁	+
IgG _{2a}	++++
IgG _{2b}	+++
IgG ₃	++
Rat	
IgG ₁	---
IgG _{2a}	---
IgG _{2b}	---
IgG _{2c}	+
POLYCLONAL	
Rabbit	++++
Cow	++
Horse	++
Goat	-
Guinea pig	++++
Sheep	+/-
Pig	+++
Rat	+/-
Mouse	++
Chicken	---
Human IgG	++++
Human IgM	---
Human IgD	---
Human IgA	---

--- (weak or no binding) → ++++ (Strong binding)

PROCEDURE

PURIFICATION OF IgG MOLECULES

1. User Supplied Materials

- Buffers: see Section 2 below.
- Disposable column with frits and reusable caps. KPL recommends Pharmacia Biotech PD-10 empty disposable columns (Catalog No. 17-0438-01) or equivalent.

2. Buffer Preparation

- Wash/Binding Buffer:** KPL Catalog No. 50-70-01 or prepare 0.1 M Sodium phosphate, 0.15 M NaCl, pH 7.4.
- Elution Buffer:** KPL Catalog No. 50-68-01 or prepare 0.2 M Glycine, pH 3.0 ± 0.15.
- Storage Buffer:** KPL Catalog No. 50-69-01 or prepare 0.01 M NaH₂PO₄, 0.15M NaCl, 2.7 mM KCl, pH 7.4, 20% ethanol.

- Sample Preparation:** To insure proper ionic strength and pH are maintained for optimal binding, it is necessary to dilute serum samples, ascites fluid or tissue culture supernatant at least 1/1 with binding buffer. Alternatively, the sample may be dialyzed overnight against wash/binding buffer. KPL recommends using a 12,000 MW cutoff dialysis tubing with at least 2 buffer exchanges. Remove any particulate matter from the sample by centrifugation or filtration through a 0.8 µm filter.

4. Column and Resin Preparation:

- Pour 20% Ethanol in the bottom of a petri dish or in a flat bottomed container. Float the frit on top of the ethanol. Using the large round end of a 1 mL pipette tip, press the frit firmly into the ethanol to force air out. Repeat this step until the frit is completely wet.
- Push the frit into the barrel of the column until it rests firmly on the bottom.
- With the cap removed, clip the end of the column to create a hole to allow liquid to flow through.
- Wash the frit with 5 column volumes of 1X Wash/Binding Buffer.
- Prepare a 1/1 suspension of resin in 1X Wash/Binding buffer. The required amount of agarose per mg of immunoglobulin being purified can be estimated by the binding capacity.

Recommended Column Volumes:

Antibody Source	Recommended bed volume (mL) per mL sample
Immune Serum	2 mL
Tissue Culture Supernatant (with 10% fetal bovine serum)	0.2 mL
Tissue Culture Supernatant (serum-free)	0.01 mL
Ascites Fluid	2 mL

- Pour slurry into column. Allow column to flow by gravity to pack the column bed.
- Equilibrate the packed affinity resin with 10 column volumes (CV) of the wash/binding buffer (e.g. if the packed bed is 1 mL, equilibrate with 10 mL wash/binding buffer).

4. Sample Purification:

- Gently apply sample to the column by layering onto the top of the resin. Be careful not to disturb the bed surface.
- Wash column with 10 CV of wash/binding buffer, or until the absorbance of eluate at 280 nm approaches the background level.
- Before beginning the elution step, set up enough tubes to collect the entire elution volume as 1 mL fractions (4 CV will be used to elute the antibody). To each collection tube add 240 µL 5X Wash/Binding Buffer. To elute the antibody, gently add 1 mL 1X Elution Buffer to the top of the resin collecting the eluate in a prepared collection tube. Repeat until the entire volume has been collected up to 4 column volumes. **Note:** If the eluate is to be collected in a single bulk volume, add 240 µL 5X Wash/Binding Buffer per mL Elution Buffer to the collection vessel before starting the elution. Elution of bound immunoglobulin can be monitored by absorbance at 280 nm, if desired.

- Column Regeneration:** Once the sample has been eluted, wash the affinity matrix with 2 CV of elution buffer. Re-equilibrate the column with at least 10 CV of 1X Wash/Binding Buffer. When column is equilibrated, pH of eluate will be the same as that of the Wash/Binding Buffer.

- Clean-in-Place:** With certain applications, substances which contain denatured proteins or lipids do not elute in the regeneration procedure. The following steps can be taken to clean the column:
 - To remove strongly bound hydrophobic proteins, lipoproteins and lipids, wash the column with a non-ionic detergent (e.g. 0.1% Triton X-100) at 37°C, with a contact time of ~1 minute.
 - Immediately re-equilibrate the column with 5 - 10 CV of 1X Wash/Binding Buffer.
 - As an alternative, wash the column with 70% ethanol. Allow the column to stand for 12 hours. Re-equilibrate the column with 5 - 10 CV of 1X Wash/Binding Buffer.
 - To remove precipitated or denatured substances, wash the column with 2 CV of 6M guanidine hydrochloride. Immediately re-equilibrate the column with 5 - 10 CV of 1X Wash/Binding Buffer (see step 5).

7. **Resin Storage:** Store affinity matrix in storage buffer at 2 - 8°C. **Do not** store the matrix frozen or at room temperature. The matrix can be stored in the column by sealing the outlets or remove from the column and stored as a slurry.

IMMUNOPRECIPITATION

For immunoprecipitation protocols, see references 3 - 5.

PRODUCT SAFETY AND HANDLING

See MSDS (Material Safety Data Sheet) for this product.

REFERENCES

1. Surolia, A., Pain, D. and Khan, M.I., (1982). *Trends Biochem. Sci.*, 7, 74 - 76.
2. Harlow, E. and Lane, D. eds. (1988). Antibodies, A Laboratory Manual. Cold Spring Harbor Laboratory, N.Y., 617 - 618.
3. Langone, J.J., (1982). *J. Immunological Methods*, 55, 277 - 296.
4. Lindmark, R., Thoren-Tolling, K., Sjoquist, J., (1983). *J. Immunological Methods*, 62, 1 - 13.
5. Thurston, C.F. and Henley, L.F., (1988). *in* Walker, J.M., ed. Methods in Molecular Biology, Vol. 3- New Protein Techniques. Humana Press: Clifton, N.J., 149 - 158.

RELATED PRODUCTS

Protein A Agarose Kit	Catalog No. 553-50-00
Protein G Agarose Kit	Catalog No. 553-51-00
Protein G Agarose	Catalog No. 223-51-01

See KPL's catalog for a complete list of antibodies, substrates and western blot kits.

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