

KPL Protein A Agarose

<u>Item No.</u>	<u>Size</u>
5710-0005 (223-50-01)	5 mL

DESCRIPTION

KPL 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

KPL Protein A Agarose is supplied in a volume of 7 mL consisting of 5 mL KPL 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 µm
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 μ C⁽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)

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PROCEDURE

PURIFICATION OF IgG MOLECULES

1. User Supplied Materials

- Buffers: see Section 2 below.
- Disposable column with frits and reusable caps. SeraCare recommends Pharmacia Biotech PD-10 empty disposable columns or equivalent.

2. Buffer Preparation

- Wash/Binding Buffer:** KPL Wash/Binding Buffer or prepare 0.1 M Sodium phosphate, 0.15 M NaCl, pH 7.4.
- Elution Buffer:** KPL Elution Buffer or prepare 0.2 M Glycine, pH 3.0 \pm 0.15.
Storage Buffer: KPL Storage Buffer 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. SeraCare 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 μ m 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 KPL 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.

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7. **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 KPL 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 KPL 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 KPL Wash/Binding Buffer (see step 6).
8. **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 SDS (Safety Data Sheet) for this product.

REFERENCES

- Surolia, A., Pain, D. and Khan, M.I., (1982). *Trends Biochem. Sci.*, 7, 74 - 76.
- Harlow, E. and Lane, D. eds. (1988). *Antibodies, A Laboratory Manual*. Cold Spring Harbor Laboratory, N.Y., 617 - 618.
- Langone, J.J., (1982). *J. Immunological Methods*, 55, 277 - 296.
- Lindmark, R., Thoren-Tolling, K., Sjoquist, J., (1983). *J. Immunological Methods*, 62, 1 - 13.
- 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**CAT. NO.**

KPL Protein A Agarose Kit	5710-0009 (553-50-01)
KPL Protein G Agarose Kit	5720-0004 (553-51-00)
KPL Protein G Agarose	5720-0002 (223-51-01)

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.