KPL Protein A Agarose Purification Kit

Item No.
5710-0009 (553-50-00)

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.

CONTENTS

The following components are found in the kit:

KPL Protein A Agarose (5 mL), 5710-0004 (223-50-00)
KPL Disposable columns (2), 5710-0010 (80-00-10)
KPL 5X Binding/Wash Buffer, 5710-0008 (50-70-01)
KPL 10X Elution Buffer, 5710-0006 (50-68-01)
KPL Storage Buffer 5710-0007 (50-69-01)

- KPL Protein A Agarose is supplied in a volume of 7 mL consisting of 5 mL Protein A Agarose in a 20% ethanol/PBS solution.
- KPL Wash/Binding Buffer is a 5X concentrate consisting of 0.5M Sodium Phosphate and 0.75M NaCl, pH 7.4.
- KPL Elution Buffer is a 10X concentrate containing 2M Glycine, pH 2.85.
- KPL Storage Buffer is ready to use at 0.01M NaH_2PO_4, 0.15M NaCl, 2.7mM KCl, pH 7.4, 20% ethanol.

STORAGE/STABILITY

Store at 2–8°C. Stable for a minimum of 1 year from date of receipt when stored at 2–8°C. Non-sterile.

NOTE: Storage of the wash/binding buffer concentrate at 2–8°C may result in the appearance of salt crystals due to decreased solubility at reduced temperatures. Before preparing the 1X working solution, warm the binding/wash buffer at 37°C until all crystals have dissolved. Mix well by swirling vigorously, then proceed as described below. Once redissolved, this will have no effect on buffer performance.

Also provided are 2 empty disposable columns with two sintered polyethylene frits with a pore size of 50 - 150 µm and reusable caps. The frits protect the agarose from running dry under gravitational buffer flow.

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

Species/Subclass	Protein A
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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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.

PROCEDURE

PURIFICATION OF IgG MOLECULES

1. **User Supplied Materials**
   a. Buffers: see Section 2 below.
   b. Disposable column with frits and reusable caps. SeraCare recommends Pharmacia Biotech PD-10 empty disposable columns or equivalent.

2. **Buffer Preparation**
   a. **Wash/Binding Buffer:** KPL Wash/Binding Buffer or prepare 0.1 M Sodium phosphate, 0.15 M NaCl, pH 7.4.
   b. **Elution Buffer:** KPL Elution Buffer or prepare 0.2 M Glycine, pH 3.0 ±0.15.
   c. **Storage Buffer:** KPL Storage Buffer or prepare 0.01 M NaH_2PO_4, 0.15M NaCl, 2.7 mM KCl, pH 7.4,20% ethanol.

3. **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 µm filter.

4. **Column and Resin Preparation:**
   a. 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.
   b. Push the frit into the barrel of the column until it rests firmly on the bottom.
   c. With the cap removed, clip the end of the column to create a hole to allow liquid to flow through.
   d. Wash the frit with 5 column volumes of 1X KPL Wash/Binding Buffer.
   e. 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

   f. Pour slurry into column. Allow column to flow by gravity to pack the column bed.
   g. 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).

5. **Sample Purification:**
   a. Gently apply sample to the column by layering onto the top of the resin. Be careful not to disturb the bed surface.
   b. Wash column with 10 CV of wash/binding buffer, or until the absorbance of eluate at 280 nm approaches the background level.
   c. 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 KPL 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.

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Note: If the eluate is to be collected in a single bulk volume, add 240 µL 5X KPL 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.

6. **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 KPL Wash/Binding Buffer. When column is equilibrated, pH of eluate will be the same as that of the KPL Wash/Binding Buffer.

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:

   a. 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.
   b. Immediately re-equilibrate the column with 5 – 10 CV of 1X KPL Wash/Binding Buffer.
   c. As an alternative, wash the column with 70% ethanol. Allow the column to stand for 12 hours.
   d. Re-equilibrate the column with 5 - 10 CV of 1X KPL Wash/Binding Buffer.
   e. 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

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	CAT. NO.
KPL Protein A	5710-0005 (223-50-01)
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.