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** [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:

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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.