KPL Protein G Agarose Purification Kit

Item No.
5720-0004 (553-51-00)

DESCRIPTION

KPL Protein G Agarose consists of recombinant protein G, which is produced in *E. coli* and after purification, is covalently immobilized onto 4% cross-linked agarose beads. KPL Protein G agarose is suitable for the isolation of IgG antibodies using column or immunoprecipitation methods. DNA sequencing of native protein G (from Streptococcal group G) has revealed two IgG-binding domains as well as sites for albumin and cell surface binding[1 - 6]. KPL Protein G has been designed to eliminate the albumin and cell surface binding domains to reduce nonspecific binding while maintaining efficient binding of the Fc region of IgGs. With the removal of these binding domains, Protein G can be used to separa

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 G Agarose is supplied in a volume of 15 mL consisting of 10 mL Protein G 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.

Also provided are two 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.

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 re-dissolved, this will have no effect on buffer performance.

Table 1. **Relative Affinity of Immobilized Protein G and Protein A for Various Antibody Species and Subclasses of polyclonal and monoclonal IgGs**[8].

Species/Subclass	Protein G	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)

PACKAGE INSERT

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5. **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.

6. **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. Re-equilibrate the column with 5 - 10 CV of 1X KPL Wash/Binding Buffer.
   d. 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 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.

PRODUCT SAFETY AND HANDLING

See SDS (Safety Data Sheet) for this product.

REFERENCES

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RELATED PRODUCTS	CAT. NO.
KPL Protein G Agarose	5720-0001 (223-51-01)
KPL Protein A Agarose Kit	5710-0009 (553-50-00)
KPL Protein A Agarose	5710-0005 (223-50-01)

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