

IgG Purification Kit (Protein L Based, Gravity Flow)

MBPP004-2NO

MBPP004-5NO

Introduction

Protein L is an immunoglobulin-binding protein that was originally isolated from the bacteria *Peptostreptococcus magnus* and provides a convenient way to separate immunoglobulins from a variety of sources. It is 36 kDa in size and can be produced recombinantly in *E. coli*. The recombinant Protein L, rProtein L, contains four immunoglobulin binding domains of the native protein and can be used for the purification of IgG, IgM, IgA and IgD containing kappa light chains from various species without interfering with the antigen binding site. Apart from antibodies Protein L is also suitable for binding of a wide range of antibody fragments such as Fabs, single-chain variable fragments (scFv), and domain antibodies (Dabs). Protein L is immobilized by means of covalent binding that avoids protein loss and allows for column re-use.

The IgG Purification Kit (Protein L Based, Gravity Flow) is based on the affinity of IgG to Protein L where rProtein L is immobilized to cross linked 4% beaded agarose resin matrix. The agarose beads can be regenerated and reused multiple times when stored properly.

The kit contains pre-packed ready-to-use columns of 2 ml rProtein L agarose resin bed in 20% ethanol.

Binding capacity: ~ 10 mg human IgG/ml resin

Ligand density: ~ 3.5 mg rProtein L/ml resin

Note: The binding capacity of Protein L Agarose Column depends upon the source of immunoglobulin. There might be deviations in binding capacities for different immunoglobulins derived from the same species.

Binding of immunoglobulins to Protein L

<u>Species/Subclass</u>	<u>Protein L</u>	<u>Species/Subclass</u>	<u>Protein L</u>
Human		Rat	
IgG ₁	++++	IgG ₁	++++
IgG ₂	++++	IgG _{2a}	++++
IgG ₃	++++	IgG _{2b}	++++
IgG ₄	++++	IgG _{2c}	++++
IgM	++++	<u>Bovine IgG</u>	-
IgA	++++	<u>Cat IgG</u>	NA
IgE	++++	<u>Chicken IgG</u>	++++
IgD	++++	<u>Dog IgG</u>	NA
Fab	++++	<u>Goat IgG</u>	-
Kappa	++++	<u>Horse IgG</u>	+/-
Lambda	-	<u>Pig IgG</u>	++++
ScFv	++++	<u>Rabbit IgG</u>	+
Mouse		<u>Sheep IgG</u>	-
IgG ₁	++++		
IgG _{2a}	++++		
IgG _{2b}	++++		
IgG ₃	++++		

Kit Contents

Product Code	Reagents	Quantity		Storage
		2 NO	5 NO	
DBCA12	Protein L Agarose Column	2 Nos	5 Nos	2 - 8°C
DS0095	10X Binding Buffer	25 ml	125 ml	2 - 8°C
DS0096	Elution Buffer	50 ml	2 X 125 ml	2 - 8°C
DS0097	Neutralizing Buffer	10 ml	50 ml	2 - 8°C

Materials needed but not provided

- Molecular Biology Grade Water (Product Code: ML064)

General Preparation Instructions

Dilute the 10X Binding Buffer to 1X using Molecular Biology Grade water before use.

Procedure for purification of IgG

- 1. Elimination of the Preservative:** Remove first the upper cap and then the lower one of the column to allow elimination of the preservative by gravity flow.
- 2. Equilibration of the Pre-packed column:** Equilibrate the column with 5X resin bed volume of Binding Buffer and allow draining the buffer through column. Do not let the resin bed dry.
- 3. Application of the Sample:** Add up to 5 ml of the sample (containing the immunoglobulin to be purified) through the top of the column. Keep sample and resin in contact for at least 15 minutes before removing the bottom cap. Collect the flow through.
- 4. Washing:** Wash the column 2 – 3 times with 5 ml of Binding Buffer to eliminate all the proteins that have not been retained in the column. Wash the column with binding buffer until the absorbance at 280 nm of the eluent reaches the baseline.
- 5. Elution of pure immunoglobulin:** Elute the bound IgG with 5 ml of Elution Buffer and collect individual 1 ml fractions of the eluate. Keep the buffer and resin in contact for at least 10 minutes before removing the bottom cap.
- 6. Neutralization of eluents:** Each 1ml eluted fraction can be neutralized by the addition of 0.15 ml of Neutralization Buffer. Assay protein concentration by measuring the absorbance at 280 nm and combine the fractions with highest absorbance.
- 7. Regeneration and Storage of Column:** Regenerate the immobilized Protein L column by washing with 10 ml of Elution Buffer. Columns can be regenerated for at least 5 times without significant loss in binding capacity. For storage wash the column with 10 ml of distilled water and store it upright in 5 ml of 20% Ethanol at 2 - 8°C.

Safety Information

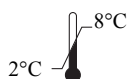
The IgG Purification Kit (Protein L based, Gravity Flow) is for laboratory use only, not for drug, household or other uses. Please refer the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Product Use Limitation & Warranty

HiMedia guarantees the performance of product in the manner described in the product literature. The IgG Purification Kit (Protein L based, Gravity Flow) is designed and sold for research and in vitro purposes only. The product is not to be used for human diagnostic or drug purposes or to be administered to humans unless expressed clearly for that purpose by the Food and Drug Administration or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in the text.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.



Storage temperature



Do not use if package is damaged



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