IgG Purification Kit (Protein A Based, Gravity Flow)

MBPP001-2NO
MBPP001-5NO

Introduction

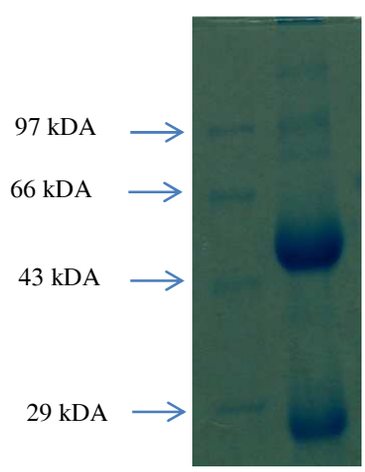
The IgG Purification Kit (Protein A Based, Gravity Flow) is based on the affinity of IgG to immobilized Protein A agarose and is used for purification of polyclonal antibodies. When a suspension (e.g. serum, ascites fluid, tissue culture supernatant) containing mixture of substances along with IgG are loaded on the column, the IgG binds to Protein A and is recovered by elution.

Protein A is a cell wall component of *Staphylococcus aureus*. It consists of a single polypeptide chain of 42 kDa which specifically binds to the Fc region of IgGs. HiMedia's Protein A is a recombinant protein, ^{r}Protein A, which contains five homologous antibody–binding domains with high affinity for subclasses of IgG from many species e.g. human, rabbit, mouse, sheep etc. and for some IgA and IgM. The binding site is located on the Fc region of immunoglobulin. Protein A is covalently coupled to agarose to prepare an affinity matrix for isolating IgGs from various species. As it binds specifically to the Fc region of IgG molecules it is used for the following:

- Purification of IgG fractions (from crude serum, ascites fluid, tissue culture supernatant)
- Isolation of antigen-antibody complex (during immunoprecipitation)

The kit contains pre-packed ready-to-use columns of 2 ml ^{r}Protein A agarose resin bed in 20% ethanol.

Binding capacity: Approximately 25 mg human IgG/ml resin
Ligand density: 3 mg ^{r}Protein A/ml resin



Lane 1: Protein Molecular Weight Marker

Lane 2: Purified IgG

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Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

Kit Contents

Product Code	Reagents	Quantity		Storage
		2 NO	5 NO	
DBCA06	Protein A 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	250 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 A 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.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	Target protein is not bound to the column.	The binding and elution conditions have to be changed.	pH, temperature and salt concentrations have to be optimized.
		Column has not been stored in recommended conditions	The recommended instructions should be followed.
		The antibody to be purified has low affinity for Protein A.	An alternative way of purification should be followed.
		Presence of proteases.	Add protease inhibitors to binding buffer.
2.	Antibody is not detected in the elution process.	The IgG subclass doesn't bind to the resin.	Use another affinity column to purify the antibody.
3.	Column flow is slow.	There are air bubbles in sample or buffers	De-gas sample and buffers before use.

Safety Information

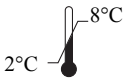
The IgG Purification Kit (Protein A based, Gravity Flow) is for laboratory use only, not for drug, household or other uses. Please refer the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Product Use Limitation & Warranty

HiMedia guarantees the performance of product in the manner described in the product literature. The IgG Purification Kit (Protein A 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

HiMedia Laboratories Private Limited,
Reg. Off: Plot No. C-40, Road No. 21Y,
MIDC, Wagle Industrial Area, Thane,
(West) 400604, Maharashtra, INDIA.
Web: www.himedialabs.com

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