IgG Purification Kit (Protein G Based, Spin Column)

MBPP002SP-5NO
MBPP002SP-25NO

Introduction

The IgG Purification Kit (Protein G Based, Spin Column) is based on the affinity of IgG to immobilized Protein G 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 G and is recovered by elution.

Protein G is a bacteria-derived cell wall protein produced by group *G streptococcus*. It is widely used for IgG purification for its high affinity for the Fc portion of most mammalian immunoglobulins. HiMedia's Protein G is a genetically engineered recombinant protein which contains three IgG-binding regions of native Protein G. The cell wall binding region, albumin binding region and other non-specific regions have been eliminated from the recombinant Protein G to ensure the maximum specific IgG binding. The recombinant Protein G has been covalently immobilized onto 4% cross-linked agarose. Immobilized Protein G is ideal for polyclonal IgG purification from mouse, human, cow, goat and sheep serum, including human IgG_3 and mouse IgG_1 isotypes.

The kit contains pre-packed ready-to-use columns of 0.2 ml ^{r}Protein G agarose resin bed in 20% ethanol.

Binding capacity: Approximately 20 mg human IgG/ml resin

Ligand density: 3 mg ^{r}ProteinG/ml resin

Kit Contents

Product Code	Reagents	Quantity		Storage
		5 NO	25 NO	
DBCA07	Protein G Agarose Spin Column	5 Nos	25 Nos	2 - 8°C
DS0095	10X Binding Buffer	20 ml	100 ml	2 - 8°C
DS0096	Elution Buffer	10 ml	50 ml	2 - 8°C
DS0097	Neutralizing Buffer	1 ml	5 ml	2 - 8°C

Materials needed but not provided

- 1X PBS (Product Code: ML116)
- Molecular Biology Grade Water (Product Code: ML064)
- Collection tubes (2.0 ml)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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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:** Equilibrate column and buffers to room temperature. Remove the lower cap of the column and place it in the 2 ml collection tube. Centrifuge at 500 X g for 1 minute to allow elimination of the preservative.

2. **Equilibration of the spin column:** Equilibrate the spin column with 0.4 ml of Binding Buffer and mix manually. Centrifuge at 500 X g for 1 minute and discard the flow through. Repeat this step once. Do not let the resin bed dry.

3. **Application of the Sample:** Close spin column outlet with cap. Add up to 0.5 ml of the sample (containing the immunoglobulin to be purified) through the top of the spin column. Close the lid and keep sample and resin in contact for at least 30 minutes before removing the bottom cap. Mix manually inverting the spin column. Centrifuge at 500 X g for 1 minute and collect the flow through.

4. **Washing:** Transfer the spin column to a new collection tube. Add 0.4 ml of Binding Buffer through the top to eliminate all the proteins that have not been retained in the column. Mix manually inverting the spin column. Centrifuge at 500 X g for 1 minute and discard the flow through. Repeat the washing step twice for a total of three washes.
   Note: Wash the spin column with Binding Buffer until the OD 280 nm of the washes reach the baseline level.
   Optional: Keep all the washes if required.

5. **Elution of pure immunoglobulin:** Transfer the spin column to a new collection tube and close the column outlet with cap. Add 0.4 ml of Elution Buffer and close the lid. Mix thoroughly for 10 minutes before removing the bottom cap. Centrifuge at 500 X g for 1 minute, collect the eluate and label it. Repeat the elution step twice for a total of three individual eluates.

6. **Neutralization of eluents:** Each 0.4 ml of eluted fraction can be neutralized by the addition of 40 µl 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 G column by washing at least 5 times with 0.4 ml of Elution Buffer. Columns can be generated for at least 5 times without significant loss in binding capacity. For storage wash the column with 5 ml of distilled water and store it upright in 0.4 ml of 20% Ethanol at 2 - 8^{o}C.

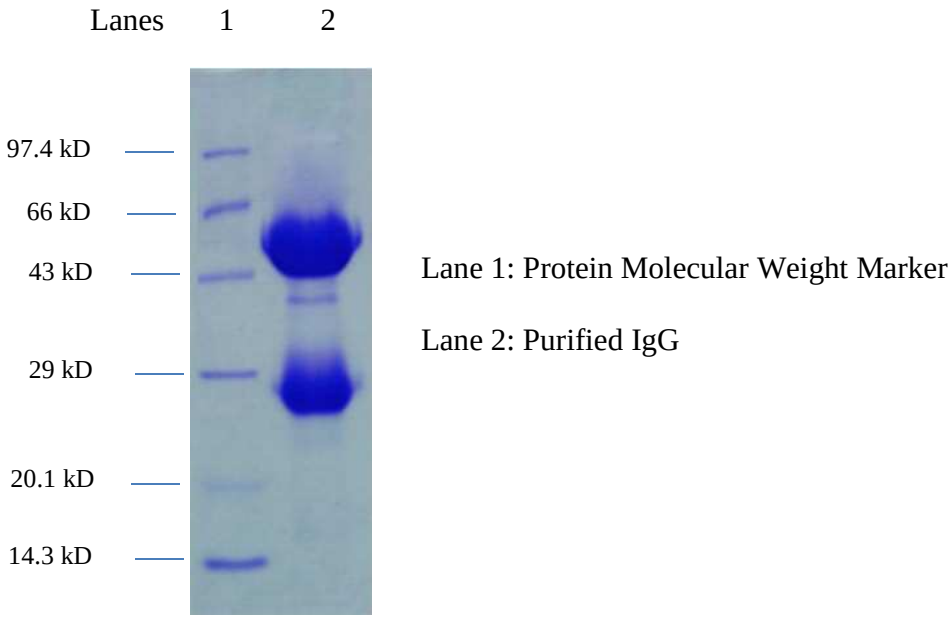


Fig1: Purified IgG on SDS-PAGE

Relative Affinity of Immobilized Protein G for various species and subclasses of polyclonal and monoclonal IgGs

Species/Subclass	Protein G
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}	++

Species/Subclass	Protein G
Polyclonal	
Rabbit	+++
Cow	++++
Horse	++++
Goat	++
Guineapig	++
Sheep	++
Pig	+++
Rat	++
Mouse	++
Chicken	+
HumanIgG	++++
HumanIgM	+
HumanIgD	+
HumanIgA	+

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 G.	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

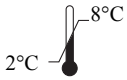
The IgG Purification Kit (Protein G Based, Spin Column) is for laboratory use only, not for drug, household or other uses. Please refer the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Product Use Limitation & Warranty

HiMedia guarantees the performance of product in the manner described in the product literature. IgG Purification Kit (Protein G Based, Spin Column) 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's, 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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