IgG Purification Kit (Protein A Based, Spin Column)

MBPP001SP-5NO
MBPP001SP-25NO

Introduction

The IgG Purification Kit (Protein A Based, Spin Column) 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 in 20% ethanol.

Binding capacity: Approximately 25 mg human IgG/ml resin

Kit Contents

Product Code	Reagents	Quantity		Storage
		5 NO	25 NO	
DBCA09	Protein A 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: ML024)
- Collection tubes (2.0 ml)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)

ISO 9001-2008 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 A column by washing at least 3 times with 0.4 ml of Elution Buffer. Columns may be

generated for at least 3 - 4 times without significant loss in binding capacity. For storage wash the column with 10 ml of distilled water and store it upright in 0.4 ml of 1X PBS (Phosphate Buffered Saline) at 2 - 8^{o}.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	Target protein is not bound to the column	Conditions in binding or elution are not the optimum ones.	Optimize pH, flow, temperature and salt concentration.
		Channels have formed in column bed so loaded sample runs through column without interacting with Protein A.	Re-pack column.
		Column has not been stored in recommended conditions alter previous usage.	Always follow the recommended instructions.
		The antibody to be purified has low affinity with Protein A.	Look for bibliography on the subject and, if that observation is true, try an alternative way of purification.
		Presence of proteases.	Add protease inhibitors to sample loading/wash buffer. Work at lower temperatures (such as 4^{o}C) to minimize degradation.
2.	The antibody is degraded.	Antibody can be unstable in elution conditions.	Follow usage instructions neutralizing the fractions of the eluted antibody.
3.	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.
4.	Column flows very slowly.	There are air bubbles in sample or buffers that are blocking flow through pores.	De-gas sample and buffers used.