Protein G Agarose Resin

DS0818-5ML
DS0818-25ML

HiMedia's Protein G Agarose Resin consists of ʳProtein G covalently bound to crosslinked agarose beads and has a very high affinity for IgG (immunoglobulins). ʳProtein 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. 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. This resin is used for batch or column purifications of classes, subclasses and fragments of immunoglobulins from serum, ascites fluid, tissue culture supernatant. It provides a very stable bond that can greatly minimize leakage of the Protein G allowing for reuse of the affinity resin in several purification steps. This product is supplied as a slurry suspension of Protein G Agarose Resin in 20% ethanol. 1 ml resin corresponds to 2 ml of 50 % (v/v) Protein G Agarose slurry suspension.

Binding capacity: Approximately 20 mg human IgG/ml resin
Ligand density: 3 mg ʳProtein G/ml resin

I. Procedure for Batch Purification of IgG

1. Elimination of the Preservative: Determine the quantity of Protein G Agarose Resin needed for purification following the recommendations. Gently shake the bottle of Protein G Agarose Resin to achieve a homogenous suspension. Immediately pipette the suspension (2 ml of the original Protein G Agarose suspension per ml of resin volume required) to an appropriate tube, sediment the resin by centrifugation at 500 x g for 5 minutes and carefully decant the supernatant and discard it.
2. Equilibration of the Resin: Add 10 bed volumes of binding buffer to equilibrate the resin by mixing thoroughly to achieve a homogenous suspension. Sediment the resin by centrifugation at 500 x g for 5 minutes and carefully decant the supernatant and discard it. *NOTE*: IgG from most species binds at neutral pH. The most frequently used buffers are 25 mM Sodium phosphate/50 mM Tris-Cl/PBS.
3. Application of the Sample: Once the resin is equilibrated, the sample containing the immunoglobulin for purification is applied. In some cases a slight increase of contact time may facilitate binding. Centrifuge the suspension at 500 x g for 5 minutes to sediment the resin and carefully decant the supernatant and discard it. Collect flow through and verify that immunoglobulin has bound.

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

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4. Washing of the Resin: Wash the resin by adding 10 ml be volumes of wash buffer. Invert to mix and centrifuge the suspension at 500 x g for 5 minutes to sediment the resin. Carefully decant the supernatant and discard it. Repeat the washing step twice.
5. Elution of Pure IgG: Add 1 bed volume of elution buffer to the resin. Mix thoroughly for 10 minutes at room temperature. Sediment the resin by centrifugation at 500 x g for 5 minutes and carefully decant or pipette the supernatant in a new tube and store on ice. Repeat the elution step twice or more and pool the fractions. *NOTE*: Elution is normally achieved at reduced pH and depending on the sample it may be necessary to decrease pH below 3.0. Most immunoglobulins are eluted in 100 mM Glycine/100 mM Citric acid buffer, pH 3.0.

II. Procedure for Gravity Purification of IgG

1. Elimination of the preservative: Determine the quantity of Protein G Agarose resin needed for purification. Gently shake the bottle of resin to achieve a homogenous suspension. Immediately pipette sufficient suspension to an appropriate empty column. 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: Close the bottom cap and add the sample containing the containing the immunoglobulin to be purified through the top of the column. Close the top cap and keep sample and resin in contact for at least 45 - 60 minutes before removing the bottom cap. Collect the flow through.
4. Washing: Close the bottom cap. Add 5X bed volumes of the wash buffer through the top to eliminate all the proteins that have not been retained in the column. Close the top cap and mix manually inverting the column. Remove the bottom cap and discard the flow through. Repeat the step twice. *NOTE*: Wash the column until the absorbance at 280 nm of the eluent reaches the baseline. **Optional:** Keep all the washes if required.
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 G 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^{o}C.

III. Procedure for Spin Purification of IgG

1. Elimination of the preservative: Determine the quantity of Protein G resin needed for purification. Gently shake the bottle of resin to achieve a homogenous suspension. Immediately pipette 100 µl of the original suspension to the empty spin column. 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 the pure protein: 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 3 times with 0.4 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 5 ml of distilled water and store it upright in 0.4 ml of 20% Ethanol at 2 - 8^{o}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 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

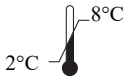
The HiMedia's Protein G Agarose Resin is for laboratory use only, not for drug, household or other uses. Please refer the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Product Use Limitation & Warranty

HiMedia guarantees the performance of product in the manner described in the product literature. Protein G agarose resin 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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