Glutathione Agarose Resin

DS0819-5ML

DS0819-25ML

Glutathione agarose Resin contains glutathione, immobilized on agarose and is used for rapid purification of glutathione S-transferase (GST) or glutathione binding proteins. For the purification of recombinant proteins additional amino acids or a whole protein is often added and is known as fusion tags. One of the most common fusion tag is glutathione *S*-transferase (GST) which can be added to a protein of interest to purify it. GST protein has a strong binding affinity for glutathione, immobilized on agarose, and when a mixture of proteins is added to this matrix, the protein of interest (tagged to GST) binds to the glutathione ligand and impurities are removed by washing with binding buffer. As a result the protein is isolated from the rest. Finally, the beads are treated with reduced glutathione (free) which competitively detaches the interaction between immobilized glutathione and the GST-tagged protein of interest from the beads which results in a purified protein.

This product is supplied as a 75% aqueous suspension (v/v) in 20% ethanol, is used for rapid purification of recombinant proteins fused to GST and expressed in bacteria.

Binding capacity: 8 mg of GST tagged protein/ml resin.

I. Procedure for Batch Purification of GST-Tagged Proteins

1. Elimination of the Preservative: Determine the quantity of Glutathione Agarose Resin needed for purification following the recommendations. Gently shake the bottle of Glutathione Agarose Resin to achieve a homogenous suspension. Immediately pipette the suspension (1.33 ml of the original Glutathione 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.
3. Application of the Sample: Once the resin is equilibrated, the sample containing the fused protein 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. *NOTE*: Binding Buffer – Phosphate Buffered Saline
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 protein: 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.

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Note: It is possible that a significant amount of GST-tagged protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among GST-tagged proteins. Eluates should be monitored by Bradford assay, SDS-PAGE or by measuring the absorbance at 280 nm.

II. Procedure for Gravity Purification of GST-Tagged Proteins

1. Elimination of the preservative: Determine the quantity of GST 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 GST-tagged protein to be purified (clarified *E. coli* lysate) 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 the pure protein: Close the bottom cap and add the elution buffer through the top. Close the top cap and mix manually inverting the column thoroughly for 15 minutes. Let the resin settled, remove the bottom cap and collect the eluate in a new tube and store on ice. Repeat the elution step twice and pool the collected eluates.

   Note: It is possible that a significant amount of GST-tagged protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among GST-tagged proteins. Eluates should be monitored by Bradford assay, SDS-PAGE or by measuring the absorbance at 280 nm.

III. Procedure for Spin Purification of GST-Tagged Proteins under native conditions

1. Elimination of the preservative: Determine the quantity of GST Agarose 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 and add the sample containing the GST-tagged protein to be purified (clarified *E. coli* lysate) 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 washing 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 column until the absorbance at 280 nm of the eluent reaches the baseline.

   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.

   Note: It is possible that a significant amount of GST-tagged protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among GST-tagged proteins. Eluates should be monitored by Bradford assay, SDS-PAGE or by measuring the absorbance at 280 nm.

Additional Information:

If the expressed proteins form inclusion bodies then the purification procedure has to be carried out in denaturing condition using 8M Urea or 6M Guanidine hydrochloride.

For denaturing conditions the following buffers can be used:

Denaturing Binding Buffer: 20mM Sodium phosphate, pH 7.8, 500 mM NaCl, 8M Urea

Denaturing Wash Buffer: 20mM Sodium phosphate, pH 6.0, 500 mM NaCl, 8M Urea

Denaturing Elution Buffer: 20mM Sodium phosphate, pH 4.0, 500 mM NaCl, 8M Urea

Regeneration and Storage of Column: During the life of the resin, it may lose binding points because some protein is retained. Hence a loss of the binding capacity may be observed in successive cycles. To return to the starting state, regeneration may be necessary. Regeneration consists of the complete elimination of the retained protein. In general, column regeneration is always necessary when changing proteins. When continuing with the same protein it is recommended to do regeneration when an appreciable reduction in the yield is observed. The frequency of these stages varies with the protein and the conditions used:

I. Wash the column with 10X bed volumes of Regeneration Buffer I first and then with Regeneration Buffer II.

II. Repeat these washes twice and finally wash with 5X bed volumes of Binding Buffer.

III. For storage wash the column with additional bed volumes of 20% Ethanol and store at 2-8°C.

Columns can be regenerated for at least 5 times without significant loss in binding capacity.

Note:

1. Regeneration Buffer I (100 mM Tris-HCl, 0.5 M NaCl, pH 8.5)
2. Regeneration Buffer II (100 mM Sodium acetate, 0.5 M NaCl pH 4.5)

Troubleshooting Guide:

Sr. No.	Problem	Cause	Solution
1.	Protein yield is low	Problems with vector construction.	Ensure that protein and tag are in frame.
		Poor protein expression.	Optimize bacterial expression conditions.
		Extraction may be insufficient.	Check extraction conditions (lysozyme, sonication).
2.	Target protein does not bind efficiently	Concentration of fusion protein is too low.	Concentrate the sample. Yield depends upon the protein concentration.
		Absence of reducing agents.	By adding DTT to the lysis buffer before cell lysis significantly increase binding of fusion proteins.
		Inadequate binding conditions.	Check the conditions.
		Column capacity exceeded.	Apply less fused protein to the column.
3.	Poor protein purity	Degradation of GST fusion protein.	Add protease inhibitors.
		There are air bubbles in sample or buffers that are blocking flow through pores.	De-gas sample and buffers used.

Safety Information

The HiMedia's Glutathione 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. Glutathione 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.

Please refer disclaimer Overleaf.

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.

Symbols

	Manufacturer		Do not use if package is damaged
LOT	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)	REF	Catalogue number

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