GST-Tagged Bacterial Protein Purification Kit (Spin Column)

MBPP003SP-5NO
MBPP003SP-25NO

Introduction: Protein purification is an essential pre-requisite for proteomics. Protein purification procedures can vary from simple one-step precipitation procedures to large scale production processes. The most accepted of these methods is affinity purification where the protein of interest is purified through its specific binding properties to an immobilized ligand. Affinity tags are very efficient tools to purify heterologous proteins from different sources because the single step purification process involves mild elution conditions and do not interfere with the structure and function of the recombinant protein. 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.

HiMedia's GST-Tagged bacterial Protein Purification Kit (Spin Column) is used for rapid purification of glutathione S-transferase (GST) or glutathione binding proteins.

The kit contains pre-packed ready-to-use columns of 0.2 ml Glutathione agarose resin bed in 20% ethanol.

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

Kit Contents:

Product Code	Reagents	Quantity		Storage
		5 NO	25 NO	
DBCA11	Glutathione Agarose Spin Column	5 Nos	25 Nos	2 - 8°C
DS0102	Binding Buffer	20 ml	100 ml	2 - 8°C
DS0103	Buffer for Elution	15 ml	75 ml	2 - 8°C
DS0104	Reduced Glutathione	5 X 60 mg	25 X 60 mg	2 - 8°C

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

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

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

General Preparation Instructions

1. Dilute the 10X Binding Buffer to 1X using Molecular Biology Grade water before use.
2. **Preparation of Elution Buffer:** Add 2 ml of Buffer for Elution (DS0103) to a single vial of Reduced Glutathione for one experiment. Before starting the experiment, bring the final volume to 20 ml using the same Buffer for Elution (DS0103).

Procedure for purification of GST-Tagged Protein

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 0.5 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 sample containing the GST-tagged protein to be purified (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 0.5 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.
Note: Always use fresh Elution Buffer while performing the protocol.

6. **Regeneration and Storage of Column:** During purification procedure the column resin loses its binding capacity in successive cycles as always some proteins are retained. For this reason regeneration is very essential. After use regenerate the immobilized GST column by washing with 10X bed volumes of Regeneration Buffer I first and then with Regeneration Buffer II. Repeat these washes twice and finally wash with 5X bed volumes of Binding Buffer. For storage wash the column with additional bed volumes of 20% Ethanol and store at 2 - 8^{o}C. Columns can be regenerated for at least 5 times without significant loss in binding capacity.

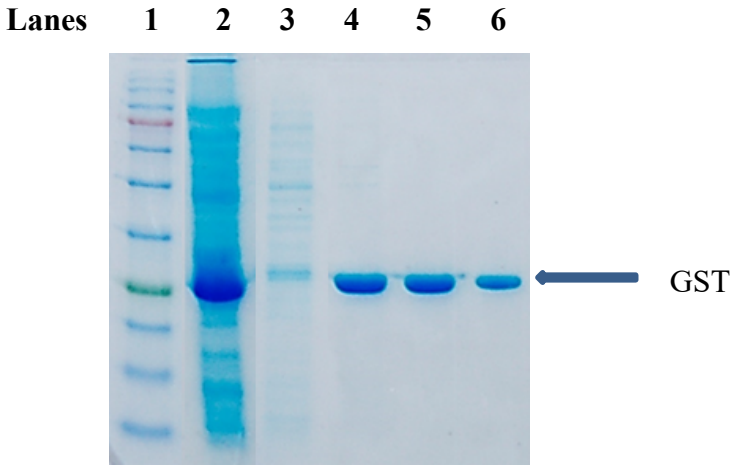

Fig1: Purified GST on SDS-PAGE

Lane 1: Marker
Lane 2: Cell extracts (expressing GST protein)
Lane 3: Flow-through
Lane 4: Eluate 1
Lane 5: Eluate 2
Lane 6: Eluate 3

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 GST-Tagged bacterial Protein Purification Kit (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. GST-Tagged bacterial Protein Purification Kit (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, 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
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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