GST-Tagged Bacterial Protein Purification Kit (Gravity Flow)

MBPP003-2NO

MBPP003-5NO

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 (Gravity Flow) is used for rapid purification of glutathione S-transferase (GST) or glutathione binding proteins.

The kit contains pre-packed ready-to-use columns of 2 ml Glutathione agarose resin bed in 20% ethanol.

Binding capacity: 8 mg of GST tagged protein/ml gel

Kit Contents:

Product Code	Reagents	Quantity		Storage
		2 NO	5 NO	
DBCA10	Glutathione Agarose Column	2 Nos	5 Nos	2 - 8°C
DS0102	Binding Buffer	25 ml	125 ml	2 - 8°C
DS0103	Buffer for Elution	50 ml	250 ml	2 - 8°C
DS0104	Reduced Glutathione	2 X 60 mg	5 X 60 mg	2 - 8°C

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

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Materials needed but not provided

- 1X PBS (Product Code: ML116)
- Molecular Biology Grade Water (Product Code: ML064)
- Collection tubes (2.0 ml)
- 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 one vial of Reduced Glutathione for one experiment. Dilute it to 1X with Buffer for Elution (DS0103) before the experiment.

Procedure for purification of GST-Tagged Protein

1. **Elimination of the Preservative:** 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 30 minutes before removing the bottom cap. Collect the flow through.

4. **Washing:** Close the bottom cap. Add the binding buffer (5X bed volumes) 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 with binding buffer until the absorbance at 280 nm of the eluent reaches the baseline.

5. **Elution of the pure protein:** Close the bottom cap and add the elution buffer (1X bed volume) through the top. Close the top cap and mix manually inverting the column thoroughly for atleast 10 minutes. Let the gel settled, remove the bottom cap and collect the eluate in a new tube and store on ice. Repeat the elution step twice.

6. **Regeneration and Storage of Column:** After use, regenerate the resin by washing first with 20 ml of Regeneration Buffer I and then with Regeneration Buffer II. These wash cycles have to be repeated twice. Finally, the column is washed with 10 ml of Binding Buffer. Columns can be regenerated for at least 5 times without significant loss in binding capacity. For storage keep the column upright in 5 ml of 20% Ethanol at 2 - 8°C.

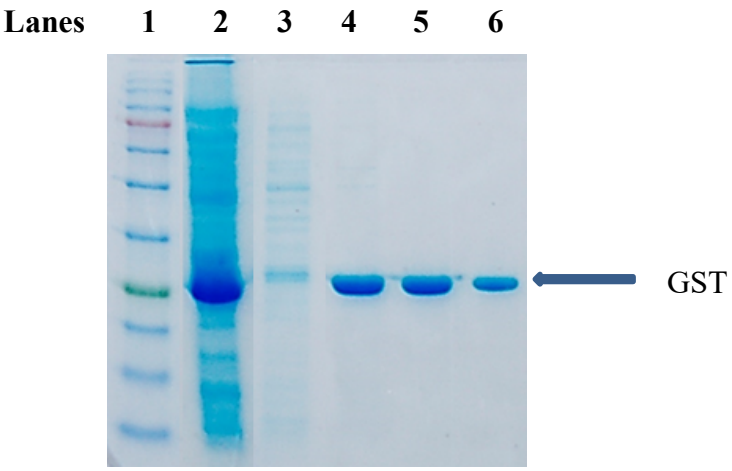


Fig1: Purified GST on SDS-PAGE

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.
4.	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 (Gravity Flow) 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.

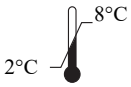
Product Use Limitation & Warranty

HiMedia guarantees the performance of product in the manner described in the product literature. GST-Tagged bacterial Protein Purification Kit (Gravity Flow) 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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