



200PR-02

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A Geno Technology, Inc. (USA) brand name

HOOK™ GST Protein Purification (Bacteria)

For the purification of GST-tagged proteins from bacteria

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ITEMS SUPPLIED Cat. # 786-641

Bacterial PE-LB™	100ml
PE LB™-Lysozyme	1ml
Glutathione Resin Columns*	5
GST Binding/Wash Buffer	200ml
Glutathione	2 vials

* Glutathione Resin Columns contain 1ml prepacked resin in 0.05% sodium azide.

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival, store PE LB™-Lysozyme at -20°C, resin refrigerated at 4°C (DO NOT FREEZE), and all other components may be stored at room temperature. The kit components are stable for 1 year when stored and used as recommended.

INTRODUCTION

HOOK™ GST Protein Purification kit allows for the purification of soluble, GST tagged protein from bacterial cultures. The bacteria are first lysed with Bacterial PE LB™ and PE LB™-Lysozyme to release total soluble protein, whilst maintaining the structure and activity of the protein. The GST tagged protein is purified by affinity chromatography by passing clarified lysate through prepacked columns.

Bacterial- PELB™ kit has been developed for the extraction of soluble proteins from bacterial cells. It is a proprietary improvement on the lysozyme based lysis, which allows extraction of soluble proteins and concurrent removal of nucleic acids (DNA & RNA) released during cell lysis. The Bacterial-PE LB™ lysis eliminates viscosity build-up, allowing effective clarification with lower centrifugal force.

HOOK™ GST Protein Purification kit is optimized to yield up to 10mg/250ml culture of soluble GST tagged protein, with a purity of 80-90%, dependent on expression levels, resin type, conformation and solubility characteristics of the protein.



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PREPARATION BEFORE USE

- I. Prior to using the HOOK™ GST Protein Purification kit, it is recommended that an estimation of the expression and solubility levels of your protein is performed. Express protein as normal and lyse with the Bacterial PE-LB™ reagents, clarify by centrifugation and view on a SDS polyacrylamide gel.
- II. An inherent problem with recombinant protein expression is solubility. Some proteins expressed in bacteria are insoluble and are localized to inclusion bodies. The supplied Bacterial PE LB™ can isolate inclusion bodies (see Additional Protocols) and these can be solubilized with our Inclusion Body Solubilization (IBS) Buffer (Cat. # 786-183) or commonly used denaturants (8M Urea or 6M Guanidine). The resulting solubilized proteins can be used with this kit, however denaturants and reducing agents may be needed in the buffers to maintain the proteins solubility.
- III. To maintain the integrity of your recombinant protein, it is recommended that a protease inhibitor cocktail is used throughout the purification process. We recommend *Recom* ProteaseARREST™ (Cat. # 786-376), a protease inhibitor cocktail specific designed for purifying recombinant proteins from bacteria, or ProteaseARREST™ (Cat. # 786-108), a general protease inhibitor that is supplied with *optional* EDTA.
- IV. The resin and buffers should be allowed to equilibrate to room temperature before beginning the purification.
- V. *GST Elution Buffer*: Dissolve one vial of glutathione in 50ml GST Binding/Wash Buffer to give a final concentration of 10mM. For long term storage, store at -20°C. We recommend freezing in smaller aliquots to limit the amount of freeze/thaws and potential oxidation of the glutathione.

ADDITIONAL MATERIALS

- ❖ Centrifuge and centrifuge tubes for harvesting 200ml bacterial culture
- ❖ Wide-bore pipette tips for dispensing the resin slurry
- ❖ Micro-centrifuge
- ❖ 15ml centrifuge tubes

PROTOCOL

1. Harvest the bacterial cells from 250ml culture bacterial culture (OD₆₀₀ 1.5-3.0) by centrifugation at 5,000xg for 10 minutes. Discard the supernatant.
NOTE: If using a frozen bacterial pellet, ensure the pellet is completely thawed before starting.
2. Pellet bacterial cells (bacterial culture, OD₆₀₀ 1.5-3.0) by centrifugation at 5,000xg for 10 minutes.
3. Resuspend the bacterial pellet in 10ml Bacterial PE LB™ by either vortexing or pipetting until a homogenous suspension is achieved.
*NOTE: If using, add your protease inhibitor cocktail to the suspension at this point.
For *Recom* ProteaseARREST™ (Cat. # 786-376) or ProteaseARREST™ (Cat. # 786-108), add 80µl.*
4. Vortex the PE LB™-Lysozyme and add 400µl PE LB™-Lysozyme to the homogenous suspension and gently mix by inverting the tube a few times. Incubate the suspension at 37°C for 30-60minutes to achieve efficient bacterial lysis.
5. Follow incubation, vortex for 30 seconds and then separate the soluble proteins from the insoluble by centrifugation at 25,000xg for 15 minutes. Transfer the clarified lysate to a 15ml conical centrifuge tube.
6. Place the capped column in a 15ml centrifuge tube and briefly centrifuge at 1,000g for 1 minute to establish the resin bed.
7. Uncap the resin column and allow the preservative to drain out by gravity.
8. Add 2 x 5ml Bacterial PE LB™ to the resin and allow to flow through.
9. Apply the clarified bacterial lysate to the column (2 x 5ml) and allow to flow through.
NOTE: We recommend saving the flow through to monitor the binding efficiency by SDS-PAGE.

10. Wash the column with 3 x 5ml Wash Buffer.
NOTE: We recommend saving the flow throughs separately to monitor the washing efficiency by SDS-PAGE.
11. Elute the GST tagged protein by adding 2 x 3ml Elution buffer and collecting the fractions that emerge.
12. The elution of the protein can be monitored by absorption at 280nm, by assaying with a protein assay (CB-X™ Protein Assay (Cat. # 786-12X) or by SDS-PAGE analysis. We recommend Tube-O-DIALYZER™ for buffer exchange and removal of excess imidazole.

ADDITIONAL PROTOCOLS: Inclusion Body Isolation

1. Harvest the bacterial cells from 250ml culture bacterial culture (OD₆₀₀ 1.5-3.0) by centrifugation at 5,000xg for 10 minutes. Discard the supernatant.
NOTE: If using a frozen bacterial pellet, ensure the pellet is completely thawed before starting.
2. Pellet bacterial cells (bacterial culture, OD₆₀₀ 1.5-3.0) by centrifugation at 5,000xg for 10 minutes.
3. Resuspend the bacterial pellet in 10ml Bacterial PE LB™ by either vortexing or pipetting until a homogenous suspension is achieved.
*NOTE: If using, add your protease inhibitor cocktail to the suspension at this point.
For Recom ProteaseARREST™ (Cat. # 786-376) or ProteaseARREST™ (Cat. # 786-108), add 80µl.*
4. Vortex the PE LB™-Lysozyme and add 400µl PE LB™-Lysozyme to the homogenous suspension and gently mix by inverting the tube a few times. Incubate the suspension at 37°C for 30-60minutes to achieve efficient bacterial lysis.
5. Follow incubation, vortex for 30 seconds and then separate the soluble proteins from the insoluble by centrifugation at 30,000xg for 30 minutes. Transfer the clarified lysate to a 15ml conical centrifuge tube, this is the soluble proteins.
6. The pellet contains the inclusion bodies. Wash the pellet with 5ml of a 1 in 10 dilution of the Bacterial PE LB™. Centrifuge at 30,000xg for 30 minutes to pellet inclusion bodies. The resulting inclusion bodies can be solubilized with our Inclusion Body Solubilization (IBS) Buffer (see protocol for Cat. # 786-183) or commonly used denaturants (8M Urea or 6M Guanidine). Once solubilized and clarified continue at step 6 of the main protocol.

TROUBLESHOOTING

Issue	Possible Cause	Suggested Solution
Low Protein Yield	Poor expression of soluble protein	Optimize bacterial expression and growth conditions. Check expression by SDS-PAGE to confirm expression.
	Protein insoluble and enters inclusion bodies	Try to limit inclusion body formation for inducing protein expression for shorter time periods or by performing inductions at 30°C. If inclusion bodies still form, follow the additional protocol for Inclusion Body Solubilization, using our Inclusion Body Solubilization (IBS) Buffer (Cat. # 786-183)
Protein Degradation	Protein is degraded by bacterial proteases	Use a protease inhibitor cocktail that does not use metal chelators. We recommend <i>Recom ProteaseARREST™</i> (Cat. # 786-376), a protease inhibitor cocktail specific designed for purifying recombinant proteins from bacteria.
Poor Protein Purity	Poor column washing	Wash the column more than twice or try increasing the imidazole concentration.
Slow Column Flow	Column overloaded or particulates added to column	Ensure the bacterial lysate is completely clear before adding resin, if necessary centrifuge the lysate a second time

RELATED PRODUCTS

1. **HOOK™ GST Protein Spin Purification (Bacteria) (Cat. #786-640):** A spin column format GST tag protein purification kit for isolation of up to 1mg protein from a 50ml bacterial culture.
2. **Protease Arrest™ (Cat. # 786-108):** A cocktail of protease inhibitors for use during protein extraction and purification. Protease Arrest™ inhibits a broad spectrum of serine, cysteine and metalloprotease as well as calpains.
3. **Recom ProteaseArrest™ (Cat. # 786-376):** Protease inhibitor cocktail specifically formulated for inhibition of bacterial proteases during the purification of recombinant proteins. Does not use metal chelators.
4. **IBS™ Buffer (Cat. # 786-183):** Inclusion bodies solubilization buffer. For solubilization of isolated inclusion bodies prior to re-folding protein procedure.
5. **CB-X™ Protein Assay (786-005):** A protein assay that is free from interference of common laboratory agents, including reducing agents, detergents, dyes, EDTA, etc.

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