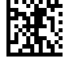

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A Geno Technology, Inc. (USA) brand name

HOOK™ 6X His Protein Spin Purification (Bacteria)

For the Purification of His-Tagged Proteins from Bacteria

(Cat. # 786-628, 786-629)

INTRODUCTION 3

ITEMS SUPPLIED 3

STORAGE CONDITION 4

SPECIFICATIONS 4

PREPARATION BEFORE USE 4

ADDITIONAL MATERIALS REQUIRED 5

PROTOCOL 5

ADDITIONAL PROTOCOL: INCLUSION BODY ISOLATION 6

TROUBLESHOOTING 7

RELATED PRODUCTS 7

INTRODUCTION

HOOK™ 6X His Protein Spin Purification kit allows for the rapid purification of soluble, 6X His 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 6X His tagged protein is purified by immobilized metal affinity chromatography (IMAC) by adding 0.4ml immobilized metal affinity resin to the clarified lysate. The resin is transferred to a convenient spin column, where it is rapidly washed and the 6X His protein is eluted with an imidazole buffer.

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™ 6X His Protein Spin Purification kit is available with either nickel chelating resin (Cat. # 786-628) or cobalt chelating resin (Cat. # 786-629) for the immobilized metal affinity chromatography. Cobalt chelating resin has a lower binding affinity for 6X His tags, compared to nickel chelating resin, which results in less non-specific binding and may result in slightly lower yields.

HOOK™ 6X His Protein Spin Purification kit is optimized to yield ~1mg/50ml culture of soluble His tagged protein, with a purity of 80-90%, dependent on expression levels, resin type, conformation and solubility characteristics of the protein.

ITEMS SUPPLIED

Description	**Cat. # 786-628**	**Cat. # 786-629**
Bacterial PE-LB™	100ml	100ml
PE LB™-Lysozyme	3 x 1ml	3 x 1ml
Nickel Chelating Resin*	10ml	-
Cobalt Chelating Resin*	-	10ml
His Binding/Wash Buffer	100ml	100ml
His Elution Buffer	100ml	100ml
Spin Column	25	25
Caps	25	25
Collection Tubes	50	50

**Nickel Chelating Resin and Cobalt Chelating Resin are supplied as 20ml 50% slurry in 20% ethanol*

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.

SPECIFICATIONS

	Nickel Chelating Resin	Cobalt Chelating Resin
Ligand Density	20-40µmoles Ni^{2+}/ml resin	20-40µmoles Co^{2+}/ml resin
Binding Capacity	~50mg/ml	~50mg/ml
Bead Structure	6% cross-linked agarose	6% cross-linked agarose

PREPARATION BEFORE USE

I. Prior to using the HOOK™ 6X His Protein Spin 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. ***PELB™ Lysozyme***: The PELB™ Lysozyme contains 40mg/ml Lysozyme (~80kU) supplemented with 800U/ml DNase and 24U/ml RNase. We recommend using the PELB™ Lysozyme at a final concentration of 0.1-1mg/ml. Higher levels of lysozyme will not improve lysis efficiency and may have an inhibitory effect.

IV. To maintain the integrity of your recombinant protein, it is recommended that a protease inhibitor cocktail is used throughout the purification process. The purification technology used is dependent on metal chelation, therefore avoid protease inhibitor cocktails that use EDTA, or other metal chelators, as an inhibitor. 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.

V. The resin and buffers should be allowed to equilibrate to room temperature before beginning the purification.

ADDITIONAL MATERIALS REQUIRED

- Centrifuge and centrifuge tubes for harvesting 50ml bacterial culture
- Wide-bore pipette tips for dispensing the resin slurry
- Micro-centrifuge
- 15ml centrifuge tubes

PROTOCOL

1. Harvest the bacterial cells from 50ml bacterial culture (OD_{600} 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. Resuspend the bacterial pellet in 2ml 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.*
3. Vortex the *PE LB™*-Lysozyme and add 5-50µl *PE LB™*-Lysozyme to the homogenous suspension and gently mix by inverting the tube a few times. Incubate the suspension at 37°C for 30-60 minutes to achieve efficient bacterial lysis.
4. 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.
5. Swirl the resin bottle to achieve a homogenous slurry and, using a wide bore pipette tip, transfer 0.8ml 50% resin slurry to the bacterial lysate. Close the tube.
6. Incubate, with shaking or rotation, for 15 minutes at room temperature.
7. Centrifuge the tube at 1,500xg for 5 minutes to pellet the resin.
8. Discard the supernatant, without disturbing the resin, and then resuspend the resin in 250µl Wash Buffer.
9. Snap off the end cap on the base of the spin column and retain. Place column in a collection tube. Transfer the resin suspension to the spin column using a wide bore pipette tip. Centrifuge the spin column assembly at 2,000xg for 2 minutes. Discard the flow through and return the spin column to the collection tube.
10. Add 500µl Wash Buffer to the spin column to wash away unbound and non-specific proteins. Incubate at room temperature for 5 minutes and then centrifuge at 2,000xg for 2 minutes. Discard the flow through and return the spin column to the collection tube.
    NOTE: If there is an issue with a large amount of non-specific proteins binding then the amount of low level of competing imidazole in the wash buffer may be increased.
11. Repeat step 11 once. Transfer the spin column to a clean collection tube.

12. Elute the 6X His tagged protein by adding 0.5ml Elution buffer to the resin and incubating at room temperature for 5 minutes. Centrifuge the spin column assembly at 2,000xg for 2 minutes. Transfer the flow through to a 1.5ml centrifuge tube and return the spin column to the collection tube. Repeat the elution three more times, storing each elution in a different 1.5ml centrifuge tube.
13. 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 PROTOCOL: INCLUSION BODY ISOLATION

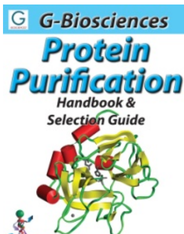
1. Harvest the bacterial cells from 50ml culture bacterial culture (OD_{600} 1.5-3.0) by centrifugation at 5,000xg for 10 minutes. Discard the supernatant.
   NOTE: *If using a frozen bacterial pellet, ensure the pellet is completely thawed before starting.*
2. Pellet bacterial cells (bacterial culture, OD_{600} 1.5-3.0) by centrifugation at 5,000xg for 10 minutes.
3. Resuspend the bacterial pellet in 2ml Bacterial PE LB™ by either vortexing or pipetting until an 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 5-50µl *PE LB™*-Lysozyme to the homogenous suspension and gently mix by inverting the tube a few times. Incubate the suspension at 37°C for 30-60 minutes 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)
	The 6X His tag may not bind column	Ensure that no metal chelators are present in the buffers. Check the sequence of the construct to ensure the tag is in frame with the protein of interest. Test for presence of the His tag by Western blotting and probing with a α-His antibody
Protein Degradation	Protein is degraded by bacterial proteases	Use a protease inhibitor cocktail that does not use metal chelators. We recommend *Recom* ProteaseARREST™ (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

Download our Protein Purification Handbook.



http://info.gbiosciences.com/complete-protein-purification-handbook

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