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HOOK™ 6X His Protein Purification (Bacteria)

For The Purification Of His-Tagged Proteins From Bacteria

(Cat. # 786-630, 786-631)

INTRODUCTION

HOOK™ 6X His Protein Purification kit allows for the 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 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™ 6X His Protein Purification kit is available with either nickel chelating immobilized metal affinity chromatography (IMAC) columns (Cat. # 786-630) or cobalt chelating IMAC columns (Cat. # 786-631). 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 Purification kit is optimized to yield >10mg/250ml 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.

ITEM(S) SUPPLIED

Description	Cat. # 786-630	Cat. # 786-631
Bacterial PE-LB™	100ml	100ml
PE LB™-Lysozyme	2 x 1ml	2 x 1ml
Nickel Chelating Columns*	5	-
Cobalt Chelating Columns	-	5
His Binding/Wash Buffer	100ml	100ml
His Elution Buffer	100ml	100ml

**Nickel Chelating Columns and Cobalt Chelating columns contain 1ml prepacked resin in 20% ethanol.*

STORAGE CONDITIONS

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

- Prior to using the HOOK™ 6X His 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.
- 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.
- ***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.
- 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.
- 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 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 (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 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.*
3. Vortex the *PE LB™*-Lysozyme and add 25-250µl *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. Place the capped column in a 15ml centrifuge tube and briefly centrifuge at 1,000g for 1 minute to establish the resin bed.
6. Uncap the resin column and allow the preservative to drain out by gravity.
7. Add 2 x 5ml Bacterial PE LB™ to the resin and allow to flow through.
8. 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.*
9. 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.*
10. Elute the 6X His tagged protein by adding 2 x 3ml Elution buffer and collecting the fractions that emerge.
11. 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

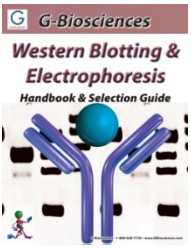
1. Harvest the bacterial cells from 250ml culture bacterial culture (OD_{600} 1.5-3.0) by centrifugation at 5,000xg for 10 minutes. Discard the supernatant.
   NOTE: If using a frozen bacterial pellet, ensure the pellet is completely thawed before starting.
2. Pellet bacterial cells (bacterial culture, OD_{600} 1.5-3.0) by centrifugation at 5,000xg for 10 minutes.
3. Resuspend the bacterial pellet in 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 25-250µl *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 Western Blotting Handbook.



http://info.gbiosciences.com/complete-western-blot-handbook--selection-guide/

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