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A Geno Technology, Inc. (USA) brand name

HOOK™ 6X His Protein Spin Purification (Yeast)

For the purification of His-tagged proteins from yeast

(Cat. # 786-632, 786-633)

INTRODUCTION

HOOK™ 6X His Protein Spin Purification kit allows for the rapid purification of soluble, 6X His tagged protein from yeast cultures. The yeast are first lysed with Yeast PE LB™ and LongLife™ Zymolyase® 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.

Yeast-PE LB™ is useful for extraction of soluble proteins from yeast cells. Yeast PE LB™ is a proprietary improvement on the Zymolyase® based spheroplast preparation and extraction of soluble proteins from yeast cells. Yeast PE LB™ is based on organic buffering agents that utilize a mild non-ionic detergent and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. A ready-to-use Zymolyase® preparation is also provided. Depending on application, additional agents such as protease inhibitors may be added into Yeast PE LB™. The proprietary combination of this reagent provides a simple and versatile method of yeast protein extraction. Yeast PE LB™ eliminates the need for laborious glass bead lysis of yeast cells.

HOOK™ 6X His Protein Spin Purification kit is available with either nickel chelating resin (Cat.# 786-632) or cobalt chelating resin (Cat.# 786-633) 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 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	786-632	786-633
Yeast Suspension Buffer	15ml	15ml
Yeast PE-LB™	100ml	100ml
LongLife™ Zymolyase®	2 x 0.5ml	2 x 0.5ml
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 25ml 50% slurry in 20% ethanol*

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival, store *LongLife*™ Zymolyase® 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 Yeast PE-LB™ reagents, clarify by centrifugation and view on a SDS polyacrylamide gel.

II. 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 *Yeast & Fungal* ProteaseARREST™ (Cat. # 786-333), a protease inhibitor cocktail specific designed for purifying proteins from yeast, or ProteaseARREST™ (Cat. # 786-108), a general protease inhibitor that is supplied with *optional* EDTA.

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

ADDITIONAL MATERIALS

- β-Mercaptoethanol
- Centrifuge and centrifuge tubes for harvesting yeast cultures
- Wide-bore pipette tips for dispensing the resin slurry
- Micro-centrifuge
- 15ml centrifuge tubes

PROTOCOL

1. Harvest the yeast cells from an overnight culture (OD_{600} 1.5-2.0) by centrifugation at 5,000xg for 10 minutes. Discard the supernatant.
   NOTE: If using a frozen cell pellet, ensure the pellet is completely thawed before starting.
2. Resuspend a maximum of 0.5ml cell pellet in 0.5ml Yeast Suspension Buffer.
   NOTE: If using, add your protease inhibitor cocktail to the suspension at this point. For Yeast & Fungal ProteaseARREST™ (Cat. # 786-333) *or* ProteaseARREST™ *(Cat. # 786-108), add 45µl.*
3. Add 5µl of β-Mercaptoethanol to the yeast suspension.
4. Vortex for 1 minute or until the cell suspension is homogeneous. Incubate the suspension for 5 minutes at 4°C. Vortex again to suspend the cells.
5. Briefly, vortex the *LongLife*™ Zymolyase® to mix the solution. Add 40µl *LongLife*™ Zymolyase® to the cell suspension. Gently mix the content.
6. Incubate the suspension at 37°C for 30-60 minutes.
7. At the end of incubation, centrifuge the suspension at 10,000x g for 5 minutes. Remove and discard the supernatant carefully, leaving the spheroplast pellet in the tube.
8. Suspend the spheroplast pellet in 4ml Yeast-*PE LB*™ Buffer. Pipette the suspension up and down a few times. Vortex periodically and incubate on ice for 30 minutes. Incubating the cells for 1-3 minutes at 37°C or a brief sonication step may further facilitate the lysis.
9. Centrifuge at 20,000x g for 30 minutes at 4°C. Transfer the clarified lysate to a 15ml centrifuge tube.
10. Swirl the resin bottle to achieve a homogenous slurry and, using a wide bore pipette tip, transfer 0.8ml 50% resin slurry to the yeast lysate. Close the tube.

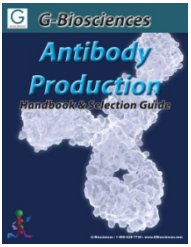
11. Incubate, with shaking or rotation, for 15 minutes at room temperature.
12. Centrifuge the tube at 1,500xg for 5 minutes to pellet the resin.
13. Discard the supernatant, without disturbing the resin, and then resuspend the resin in 250µl Wash Buffer.
14. Snap off the end cap on the base of the spin column and retain. Place the 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.
15. 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.
16. Repeat step 11 once. Transfer the spin column to a clean collection tube.
17. 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.
18. 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.

TROUBLESHOOTING

Issue	Possible Cause	Suggested Solution
Low Protein Yield	Poor expression of soluble protein	Optimize yeast expression and growth conditions. Check expression by SDS-PAGE to confirm expression.
	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 *Yeast/ Fungal* ProteaseARREST™ (Cat. # 786-333), a protease inhibitor cocktail specific designed for purifying proteins from yeast.
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 Antibody Production Handbook.



http://info.gbiosciences.com/complete-Antibody-Production-handbook

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