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A Geno Technology, Inc. (USA) brand name

HOOK™ GST Protein Purification (Yeast)

For the Purification of His-Tagged Proteins from Yeast

(Cat. # 786-643)

INTRODUCTION

HOOK™ GST Protein Purification kit allows for the purification of soluble, GST 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 GST tagged protein is purified by affinity chromatography by passing the clarified lysate through prepacked columns.

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

ITEM(S) SUPPLIED (CAT. # 786-643)

Description	Size
Yeast Suspension Buffer	15ml
Yeast PE-LB™	100ml
LongLife™ Zymolyase®	0.5ml
Glutathione Resin Column*	5
GST Binding/Wash Buffer	200ml
Glutathione	2 vials

**Glutathione Resin Columns contain 1ml prepacked resin in 20% ethanol.*

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival, store *LongLife*™ Zymolyase® at -20°C, resin columns 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.

PREPARATION BEFORE USE

1. 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 Yeast PE-LB™ reagents, clarify by centrifugation and view on a SDS polyacrylamide gel.

2. To maintain the integrity of your recombinant protein, it is recommended that a protease inhibitor cocktail is used throughout the purification process. 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.

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

4. *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 ITEM(S) REQUIRED

- 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 the cell pellet in an equal volume of Yeast Suspension Buffer. We recommend using a maximum of 1ml cell pellet.
   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. 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.

4. Briefly, vortex the *LongLife*™ Zymolyase® to mix the solution. Add 10µl *LongLife*™ Zymolyase® for every 100µl cell suspension. Gently mix the content.

5. Incubate the suspension at 37°C for 30-60 minutes.

6. 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.

7. Suspend the spheroplast pellet in Yeast-*PE LB*™ Buffer, using 2-3 times the spheroplast pellet size. 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.

8. Centrifuge at 20,000x g for 30 minutes at 4°C. Transfer the clarified lysate to a 15ml centrifuge tube.

9. Place the capped column in a 15ml centrifuge tube and briefly centrifuge at 1,000g for 1 minute to establish the resin bed.

10. Uncap the resin column and allow the preservative to drain out by gravity.

11. Add 2 x 5ml Yeast PE LB™ to the resin and allow to flow through.

12. Apply the clarified yeast 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.*

13. Wash the column with 3 x 5ml Wash Buffer.
    NOTE: *We recommend saving the flows through separately to monitor the washing efficiency by SDS-PAGE*.

14. Elute the GST tagged protein by adding 2 x 3ml Elution buffer and collecting the fractions that emerge.

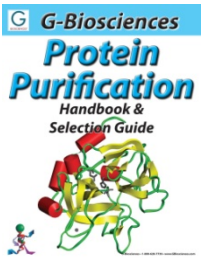
15. The elution of the protein can be monitored by absorption at 280nm, by assaying with a protein assay (CB-X™ Protein Assay (Cat. # 786-12X) or by SDS-PAGE analysis. We recommend Tube-O-DIALYZER™ for buffer exchange and removal of excess imidazole.

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 GST tag may not bind column	Check the sequence of the construct to ensure the tag is in frame with the protein of interest. Test for presence of the GST tag by Western blotting and probing with a α-GST 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 Protein Purification Handbook.



http://info2.gbiosciences.com/complete-protein-purification-handbook

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