GST-tagged Protein Purification Kit

Introduction

Glutathione-S-transferase (GST) is a highly soluble and stable 26 kDa enzyme that catalyzes the protective mechanisms of Glutathione (GSH). Many eukaryotic proteins are produced as inclusion bodies (insoluble aggregated protein), a malfunctioning protein caused by misfolding, in prokaryotic expression systems such as E. coli. It is usually painful to refold the inclusion body into a functional protein. GST is widely used as a fusion partner that promotes greater expression and solubility of the desired protein by taking advantage of its high stability and solubility to overcome this problem. Moreover, GST has a high affinity toward reduced Glutathione, its natural substrate. Therefore, GST as an affinity tag becomes a versatile tool for single-step purification of active recombinant production in a prokaryotic expression system.

Glutathione is a short peptide (Glu-Cys-Gly) that displays a high affinity toward glutathione S-transferase (GST). When the Glutathione is immobilized in a chromatography matrix such as beaded agarose or magnetic beads, the matrix can precisely capture GST-tagged protein via the affinity interaction. The GST tag can be fused to either the C- or N-terminus of a protein by inserting DNA sequence coding for the protein of interest into commercial expression vectors. The protein of interest can be cleaved off the GST tag by site-specific protease if desired. The protease site can be engineered between GST-tag and the protein of interest.

Currently, Glutathione is mainly immobilized to the traditional affinity chromatography matrices such as agarose resin or column. These solid matrices make the purification process tedious, time-consuming, unable to handle very tiny samples, and challenging to adapt to the automation system. Bioclone introduces a powerful magnetic beads-based GST–tagged protein system to overcome these problems.

BcMag™ GST-Tagged protein purification magnetic beads are magnetic microspheres covalently immobilized with a high density of Glutathione. The microspheres combine all the advantages of affinity protein purification (low costs, simplicity, high specificity, and capacity**)** and magnetic properties to perform efficient manual or automatic quick high-throughput purification. It is specially designed for the capture and purification of GST-tagged proteins from various sample types.

Workflow

The purification with magnetic microparticles is straightforward (Fig.1). Mix the microparticles with the cell lysates and incubate them with continuous rotation for a sufficient time. The beads remain suspended in the sample solution during mixing, allowing the GST-tagged protein to bind to the immobilized ligand. After incubation, the beads are collected and separated from the sample using a magnet rack. Then the ultrapure GST-tagged recombinant proteins are eluted by excess reduced Glutathione.

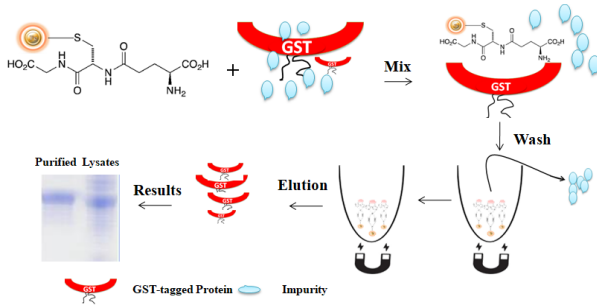

Fig1. GST-tagged protein purification

Features and Advantages

- Quick, Easy, and one-step high-throughput procedure; eliminates columns or filters or a laborious repeat of pipetting or centrifugation.
- Stable covalent bond with minimal ligand leakage
- High binding capacity, very low nonspecific binding;

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- Scalable -easily adjusts for sample size and automation;
- Reproducible results

Applications

- Investigating protein structure and function
- Preparing recombinant proteins for X-ray crystallography
- Ideal for study of protein interactions with protein or DNA
- Immunization to raise antibodies against a protein of interest.
- Effective screening protein expression even with crude cell lysates
- Microscale purification of GST-tagged proteins

Specificities	
Composition	Magnetic microsphere immobilized with Glutathione
Magnetization	~60 EMU/g
Type of Magnetization	Superparamagnetic
Effective Density	2.5 g/ml
Concentration	50 mg/ml (1x PBS)
Binding Capacity	>2mg GST /ml of Beads
Storage	Store at 4°C upon receipt

Protocol

Note

- This protocol is optimized to capture and purify GST-tagged protein from E. coli. For insoluble GST-tagged protein, we recommend refolding GST-tagged protein before purification. Creating a universal kit for protein purification is difficult because each protein has a different composition and structure. To get the best results, the user should optimize the working conditions for the purification of individual proteins based on the suggestions described in the troubleshooting section. For purification from other expression systems, optimizing the working condition is necessary.
- Recombinant protein expressed in E.coli is classified into two groups: soluble and insoluble. Soluble: The Recombinant proteins remain soluble in the cytoplasm. Insoluble: The recombinant protein is accumulated as insoluble aggregates (called inclusion bodies). The soluble target protein is found in the supernatant while inclusion bodies remain in the pellet. Practically, the solubility of the recombinant protein can be determined by the following method. Harvest the bacterial cells by centrifuge and lyse the cell. Separate the insoluble fraction of the lysed cells from the soluble fraction by centrifugation and perform an SDS-PAGE analysis.
- Before purifying the GST-Tagged fusion protein, you should equilibrate all the reagents contained in the kit at room temperature.

Materials Required

BcMag™ GST-tagged Protein Purification Magnetic Beads

Buffer

- 1x Binding/Washing Buffer (0.14 M NaCl, 10mM $Na2HPO_4$, 1.8 mM KH_2PO_4, 2.7 mM KCl, pH 7.5)
- 1x Elution Buffer (50 mM Tris-HCl, pH 8.0)

  Note: Dissolve 100 mg Glutathione (reduced) in 10 ml of 1x Elution Buffer. Prepare fresh.
- PBS Buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.5)

Equipment

- Centrifuge
- Ultrasonic processor
- Ice bucket filled with ice.
- Magnetic rack (for manual operation)

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Based on sample volume, the user can choose one of the following magnetic Racks: BcMag rack-2 for holding two individual 1.5 ml centrifuge tubes (Cat. # MS-01); BcMag rack-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Cat. # MS-04); BcMag™ rack-96 for holding a 96 ELISA plate or PCR plate (Cat. # MS-05). For larger scale purification, Ceramic magnets Block for large scale purification (6 in x 4 in x 1 in block ferrite magnet, Applied Magnets, Cat# CERAMIC-B8)

- Corning 430825 cell culture flask for large scale purification (Cole-Parmer, Cat#EW-01936-22)
- Mini BlotBoy 3D Rocker, fixed speed, small 10" x 7.5" platform w/ flat mat (Benchmark Scientific, Inc. Cat# B3D1008) or compatible

Procedure

A. Cell Extract Preparation

1. Harvest cells by centrifugation at 6000 rpm for 6 minutes, remove the supernatant and wash the pellet with 1 x PBS buffer.
2. Remove the supernatant entirely and freeze the cell pellet at -80ºC for 6 hours or overnight.
3. Thaw the cell pellets at room temperature for 15-30 minutes until they detach from the wall.
4. Suspend the cell pellets with an appropriate volume of 1x Binding/Washing Buffer (30 ul lysis buffer per 1ml bacteria culture) and incubate at room temperature for 30-60 minutes with gentle stirring.
5. Lyse cell by French press or sonication at 4°C.
   - Lyse cell by French press: Pass through French press 2-3x to ensure efficient lysis. French press should be chilled and operated at 4°C.
   - Lyse cell by Sonication: Sonicate the sample on ice using three 10-second bursts at high intensity and let the mixture cool down for 30 seconds on ice until the sample is not sticky.
6. Centrifuge at 16000 rpm for 15 minutes at 4°C.
7. Remove the supernatant containing soluble recombinant protein and transfer it to a new tube.
8. Save the cell pellet that contains insoluble protein.

- Aspirate 10 µl of the supernatant from soluble and insoluble fraction, add an equal volume of 2x SDS sample loading buffer, boil for 5 minutes and run SDS-PAGE to determine the expression level and solubility of GST-tagged protein. If protein is insoluble, refold the protein before purification.

B. Protein purification

a. Magnetic beads preparation

1. Vigorously shake the bottle until the magnetic beads become homogeneous and transfer an appropriate volume of the magnetic beads (20 ul beads** per 1ml bacteria culture) from the bottle to a new tube or flask.

   Note:

   - ***Optimize the number of beads used for each application. Too many beads will cause higher background. Insufficient beads will lead to lower yields.***
   - ***Do not allow the beads to sit for more than 2 minutes before dispensing. Resuspend the magnetic beads every 3 minutes.***
2. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack. Add ten bead-bed volumes of dH_2O_2 and mix the beads by pipetting or vortex. Again, place the tube on the magnetic rack for 1-3 minutes and remove the supernatant while the tube remains on the rack.
3. Repeat step (2) one more time.
4. Equilibrate the beads by adding ten bead-bed volumes of 1x Binding/Washing Buffer and shake it to mix them. Incubate at room temperature with continuous rotation for 2 minutes. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack. The beads are ready for purification.

b. Purification

1. Add the equilibrated beads to the cell lysates (Step A (7) and incubate on Mini BlotBoy 3D Rocker with continuous rotation for 5-10 minutes.

2. Place the tube on the magnetic rack for 1-3 minutes. Remove the supernatant while the tube remains on the rack. Add ten bead-bed volumes of 1x Binding/Washing Buffer and shake it ten times to wash the beads. Again, place the tube on the magnetic rack for 1-3 minutes and remove the supernatant while the tube remains on the rack.
3. Repeat step (2) six times.

   Note:

   - ***This step is critical to get high pure protein. It may be necessary to wash the beads more than six times for some proteins to reduce the nonspecific binding.***
   - ***Add a non-ionic detergent (0.5% Triton X 100, 0.5% Tween 20) to the binding/washing buffer, which may reduce the nonspecific binding.***
4. Elute protein with an appropriate volume of elution buffer (10 ul elution buffer per 1ml bacteria culture) by pipetting up and down 10-15 times or vortex mixer for 5 minutes.

   Note: Optimize the elution condition by adjusting the Glutathione (reduced) for each protein, and it may be necessary to elute the protein 2-3 times.
5. Collect and transfer the supernatant to a new tube.

Troubleshooting

Problem	Probable cause	Suggestion
The yield of the purified protein is too low or undetectable in eluted protein solution by SDS-PAGE.	The fusion protein forms an inclusion body.	• Grow bacteria at 14º C. • Reduce the final concentration of IPTG to 0.1mM for protein induction. • Reduce the induction time. • Properly refold the inclusion body before the purification.
	The fusion protein does not contain active GST.	• Try other fusion protein methods, such as His-Tag, to produce an alternative fusion protein. • .
	The protein does not bind to the Magnetic Beads.	• Check the pH of all the buffers and solutions.
	Harsh sonication condition denatures the fusion protein. .	• Try to use mild sonication condition or another method, such as lysozyme.
	The fusion protein does not bind to the beads.	• Add 5 mM DTT to a final concentration in Binding Buffer before cell lysis. • Check the pH of the Binding Buffer (pH should be 6.5-8.0
	The fusion protein is not efficiently eluted from beads	• Increase elution time. • Increase the concentration of Glutathione in the Elution Buffer. (Please check final pH and adjust if necessary • Adjust the pH of the Elution Buffer to 8.0-9.0 without increasing the glutathione concentration. • Add Triton X-100 (0.1%, final concentration) or Noctylglucoside (2%, final concentration) or NaCl (0.1 - 0.2 M, final concentration) to the Elution Buffer
Observe multiple bands in the eluted protein.	Degradation of the fusion protein	• Add appropriate protease inhibitor. • Use protease-deficient expression host.
	The washing condition is not optimized.	• Increase washing time and washing volume
	Some host proteins, such as chaperonins, may interact with the fusion protein.	• Increase the concentration of imidazole. • Increase washing time and washing volume
Eluted proteins lose activity	Some proteins will lose or decrease activity when fused to GST-tag.	• Try to fuse the GST tag at the other end of the protein. • Switch to other fusion tags.

Related Products	
IDA Magnetic Beads	Secreted His-tagged protein purification Kit
IDA-His-tagged protein purification Kit	Low Expression His-tagged Protein Purification Kit
GST-tagged Protein Purification Kit	

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