Purification of GST-tagged proteins using PureCube Glutathione MagBeads

Overview

This protocol describes the generation of a cleared lysate from an *E. coli* cell pellet and the subsequent purification of GST-tagged proteins under native conditions using PureCube Glutathione MagBeads. Reagent amounts given apply to 10 mL IPTG-induced bacterial culture of a well-expressed protein (approximately 10–50 mg/L).

In this protocol, cell lysis is done using lysozyme because it is an inexpensive and efficient method for cells that have been frozen. However, lysis methods using detergents (e.g., CHAPS) can also be used. The GST-tagged target protein is purified from cleared lysate under native conditions in a bind-wash-elute procedure.

Magnetic beads are well-suited to purify proteins from dilute solutions, such as cell culture or medium supernatants. In addition, magnetic bead purification is easily scalable. To minimize unspecific binding and reduce cost, the volume magnetic bead suspension used should be adjusted to the expression level of interest. See Table 1 for more details.

Please contact us if you have questions or need assistance optimizing a protocol for your application (contact@cube-biotech.com); other protocols can also be found at www.cube-biotech/protocols.

Equipment

- ☐ Ice bath
- ☐ Refrigerated centrifuge (min 10,000xg)
- ☐ Micropipettor
- ☐ Micropipetting tips
- ☐ 1.5 mL conical microcentrifuge tubes
- ☐ Magnetic holder for microcentrifuge tubes (for separation of magnetic beads)
- ☐ pH meter
- ☐ Vortex
- ☐ End-over-end shaker
- ☐ SDS-PAGE equipment
- ☐ Optional: Western Blot equipment

Materials

- ☐ Cell pellet from expression screen (e.g., from 10 mL culture)
- ☐ PureCube Glutathione MagBeads (Cube #32201)
- ☐ Sodium chloride (NaCl)
- ☐ Lysozyme
- ☐ Benzonase® nuclease (e.g. Merck Milipore #707464)
- ☐ Protease inhibitor cocktail (e.g., Roche cOmplete, #04693116001)
- ☐ Dithiothreitol (DTT)
- ☐ Glycerol
- ☐ Sodium dodecyl sulfate (SDS)
- ☐ Bromophenol blue
- ☐ Tris base
- ☐ Hydrochloric acid (HCl)
- ☐ EDTA (e.g. Cube #61262)
- ☐ Triton X-100
- ☐ Reduced Glutathione (e.g. Cube # 61033)
- ☐ Optional: Igepal CA-630 (Nonindet P40)
- ☐ Optional: Tween-20
- ☐ Optional: Adenosin triphosphate (ATP)
- ☐ Optional: $MgSO_4$
- ☐ Optional: GST Antibody (Cube Biotech #40060)

Solutions and buffers

Lysis Buffer, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Tris-HCl, pH 7.4	125 mM	121.14	0.5 M	30.29 g/500 mL	25 mL
NaCl	150 mM	58.44	5 M	146.1 g/500 mL	3 mL
DTT	1 mM	154.25	1 M	1.54 g/10 mL	100 µL
EDTA	1 mM	292.24	0.5 M	14.6 g/100 mL	200 µL
Lysozyme	1 mg/mL		100 mg/mL	1 g /10 mL	1 mL
Triton X-100	1% (v/v)		100%(v/v)		1 mL
Protease inhibitor	1x				2 tablets
Instructions: Prepare a 0.5 M Tris-HCl stock by dissolving Tris base in 400 mL deionized water, adding HCl to a pH of 7.4, and adding water to a final volume of 500 mL. Lysis buffer should always be prepared fresh.					

Wash Buffer, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Tris-HCl, pH 7.4	125 mM	121.14	0.5 M	30.29 g/500 mL	25 mL
NaCl	150 mM	58.44	5 M	146.1 g/500 mL	3 mL
DTT	1 mM	154.25	1 M	1.54 g/10 mL	100 µL
EDTA	1 mM	292.24	0.5 M	14.6 g/100 mL	200 µL
Instructions: Add water to a total volume of 100 mL. **Note**: Optimal buffer conditions may vary depending on the protein of interest. Proteins may require addition of protease inhibitor cocktail, EDTA, 1-5 mM DTT, 1% BSA, or detergents such as 0.5-1% Igepal CA-630 (Nonindet P-40) or 0.5-1% Tween-20.					

ATP Buffer (optional), 10 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Tris HCl, pH 7.4	50 mM	121.14	0.5 M	30.29 g/500 mL	1 mL
ATP	2 mM	551.14	100 mM	551 mg/1 mL	200 µL
$MgSO_4$	10 mM	120.37	1 M	1.24 g/ 1 mL	100 µL
Instructions: Add water to 10 mL. Always prepare fresh.					

Elution Buffer, 10 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Tris base, pH 7.4	125 mM	121.14	0.5 M	30.29/ 500 mL	2.5 mL
NaCl	150 mM	58.44	5 M	146.1 g/ 500 mL	300 µL
Triton X-100	0.1% (v/v)		100% (v/v)		10 µL
Reduced glutathione	50 mM	307.32			154 mg
DTT	1 mM	154.25	1 M	1.54 g/10 mL	10 µL
Instructions: Dissolve in 8 mL water, stir until the reduced glutathione is completely dissolved. Dissolve in 8 mL water, stir until the reduced glutathione is completely dissolved. Depending on the protein's requirements, set the pH to 7.4-8.0 using NaOH, then add water to 10 mL. Always prepare fresh.					

5X SDS-PAGE Buffer, 10 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Tris-HCl, pH 6.8–7.0	300 mM	121.14	1 M	12.11 g/ 100 mL	3 mL
Glycerol	50% (v/v)	–	100% (v/v)	–	5 mL
SDS	5% (w/v)	–	–	–	0.5 g
Bromophenol blue	0.05% (w/v)	–	4%	–	125 µL
DTT	250 mM	154.25	1 M	1.54 g/ 10 mL	125 µL/aliquot
Instructions: Prepare a 1 M Tris-HCl stock by dissolving Tris base in 60 mL deionized water, adding HCl to a pH of 6.8–7.0, and adding water to a final volume of 100 mL. For the SDS-PAGE Buffer, mix all components listed **except DTT** and add water to a total of 10 mL. Freeze aliquots (375 µL each) at –20 °C. Before use, add DTT to the needed single aliquots.					

Table 1. Magnetic bead suspension volumes suitable for given protein expression levels

Protein expression level	Amount of GST-tagged protein per 1 mL culture	Amount GST-tagged protein per 10 mL* culture	Volume 25% magnetic bead suspension per 10 mL culture	Minimum elution volume per 10 mL culture
<0.5 mg/L	<0.5 µg	<5 µg	2 µL*	25 µL
1 mg/L	1 µg	10 µg	4 µL*	25 µL
5 mg/L	5 µg	50 µg	20 µL	50 µL
10 mg/L	10 µg	100 µg	40 µL	100 µL
50 mg/L	50 µg	500 µg	200 µL	500 µL
***Note:** For easier handling, dilute the magnetic bead suspension in PBS buffer pH 7.4 before use.				

Procedure

1. Thaw the *E. coli* cell pellet corresponding to 10 mL bacterial culture on ice.

   Optional: Freezing the cell pellet at –20°C for 30 min prior to incubation at room temperature improves lysis by lysozyme.

2. Resuspend the cell pellet in 1 mL Lysis Buffer. Add 30 U Benzonase® (3 units/mL bacterial culture) to the lysate to reduce viscosity caused by genomic DNA.
3. Incubate for 30 min on ice, if necessary for protein stability. Otherwise, incubating at room temperature (20–25°C) may be more efficient.
4. Centrifuge the lysate for 30 min at 10,000xg and 2–8°C. Collect the supernatant.

   Note: The supernatant contains the **cleared lysate fraction.**
   We recommend to take aliquots of all fractions for SDS-PAGE analysis.

6. Resuspend the PureCube Glutathione MagBeads by vortexing. Transfer 40 µL of the 25% magnetic beads suspension into a conical microcentrifuge tube.
7. Add 500 µL Lysis Buffer to the MagBeads and mix by vortexing. Place the tube on a magnetic microtube stand until the beads are separated and discard the supernatant.
8. Pipet 1 mL of the cleared lysate onto the equilibrated MagBeads, and incubate the mixture at 4°C for 1 h on an end-over-end shaker.
9. Place the tube on the magnetic microtube stand until the beads separate and remove the supernatant.

   This is the **flow-through fraction**.

10. Remove the tube from the magnet. Add 500 µL Wash Buffer and mix by vortexing. Place the tube again on the magnetic microtube stand and allow the beads to separate. Remove the supernatant.

    These are the **wash fractions.**

11. Repeat step 10 twice.

    Optional: To remove contaminants such as chaperones, perform an additional wash step with ATP buffer.

12. Elute the GST-tagged protein using 100 µL Elution buffer (or the volume adjusted to the expression level; see Table 1)

    These are the **elution fractions.**

13. Repeat step 12. Collect each elution fraction in a separate tube.
14. Analyze all fractions by SDS-PAGE.

    Note: Do not boil membrane proteins. Instead, incubate the sample at 46°C for 30 min in preparation for SDS-PAGE analysis.

15. Optional: Perform Western Blot experiment using GST Antibody.