Purification of GST-tagged proteins using PureCube Glutathione Agarose

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 Agarose. Reagent amounts given apply to 200 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 using Glutathione Agarose under native conditions in a bind-wash-elute procedure. In this protocol, binding is performed in batch mode (in contrast to on-column binding) because it is the most efficient method, especially when the target protein is present only at low concentrations.

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.com/protocols.

Equipment

- ☐ Ice bath
- ☐ Refrigerated centrifuge for 50 mL tube (min 10,000 x g)
- ☐ 50 mL centrifuge tube
- ☐ Micropipettor
- ☐ Micropipetting tips
- ☐ Disposable gravity-flow columns with capped bottom outlet, 2 ml, (e.g. Pierce / ThermoScientific #29920)
- ☐ Optional: 15 mL conical propylene tubes (e.g. Falcon)
- ☐ pH meter
- ☐ End-over-end shaker
- ☐ UV/VIS Spectrophotometer
- ☐ SDS-PAGE equipment
- ☐ Optional: Western Blot equipment

Materials

- ☐ Cell pellet from 200 mL culture (ca. 0.5 g)
- ☐ PureCube Glutathione Agarose (Cube Biotech #32103)
- ☐ 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 Biotech #61262)
- ☐ Triton X-100
- ☐ Reduced Glutathione (e.g. Cube Biotech # 61033)
- ☐ Optional: Igepal CA-630 (Nonindet P40)
- ☐ Optional: Tween-20
- ☐ Optional: Adenosintriphosphat (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. 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.					

Procedure

1. Thaw the *E. coli* cell pellets corresponding to 200 mL bacterial culture on ice for 15 min.

   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 10 mL Lysis Buffer and pour it into a 50 mL conical centrifuge tube. If the solution is very viscous, add 3 units Benzonase® per mL *E.coli* culture volume to the lysis buffer. Alternatively or additionally, sonicate the lysate to improve cell disruption.
3. Incubate on an end-over-end shaker at room temperature for 30 min, or at 4°C for 1 h, depending on the temperature stability of the protein.
4. Centrifuge the lysate at 10.000 x g for 30 min at 2-8°C and carefully collect the supernatant without touching the pellet.

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

5. Resuspend the PureCube Glutathione Agarose by inverting the bottle until the suspension is homogeneous. Transfer 1 mL of the 50% suspension (corresponding to 500 µL bed volume) into a 15 mL conical centrifuge tube. Allow the resin to settle by gravity and remove the supernatant.

   Tip: Alternatively, resin equilibration can be performed directly in the disposable gravity flow column.

6. Add 5 mL of Wash Buffer and gently resupend the suspension to equilibrate the resin. Allow the resin to settle by gravity and remove the supernatant.
7. Add the cleared lysate prepared in step 4 and incubate at 4°C for 1 h on an end-over-end shaker.

   Tip: Alternatively, batch binding can be performed directly in a gravity flow column with closed bottom and top outlets.

8. Pour the complete suspension into a disposable gravity flow column with a capped bottom outlet.
9. Remove the bottom cap of the column and collect the flow-through.

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

10. Wash twice with 2.5 mL each of Wash Buffer.

    These are the **wash fractions.**

11. Optional: To remove contaminants such as chaperones, perform an additional wash step with ATP buffer.
12. Elute the GST-tagged protein by adding 0.5 mL Elution Buffer.

    These are the **elution fractions.**
    Optional: Incubate the resin for 15 min in Elution Buffer before collecting the eluate to increase protein yields.

13. Repeat step 11 five times, for a total of six elutions. Collect each elution fraction separately.
14. Determine the protein concentration of the elution fractions with Bradford assay, using BSA as protein standard.

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

15. Analyze all fractions by SDS-PAGE.
16. Optional: Perform Western Blot experiment using GST Antibody.