Purification of GST-tagged proteins using PureCube Glutathione Agarose and PureCube 1-step batch Mini Columns

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 10 mL IPTG-induced bacterial culture of a well-expressed protein (approximately 10–50 mg/L).
The protocol uses the revolutionary 1-step batch Mini Columns that feature the SelfSeal™ membrane technology, saving time and pipetting steps. They replace disposable gravity flow columns used in standard protocols. Volumes of up to 600 µL can be applied to a Mini Column. For larger scale experiments, 1-step batch Midi Plus Columns are available.

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. Batch binding can be done directly in the 1-step batch Mini Column to simplify the procedure.

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

- ☐ PureCube 1-step batch Mini Columns (Cube Biotech#63103)
- ☐ Ice bath
- ☐ Microcentrifuge with rotor suitable for 2 mL centrifuge tubes (min 10,000 xg)
- ☐ 2 mL centrifuge tubes
- ☐ Micropipettor
- ☐ Micropipetting tips
- ☐ pH meter
- ☐ End-over-end shaker
- ☐ UV/VIS Spectrophotometer
- ☐ SDS-PAGE equipment
- ☐ Optional: Western Blot equipment

Materials

- ☐ Cell pellet from 10 mL culture (ca. 25 mg)
- ☐ PureCube Glutathione Agarose (10 mL; 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. 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 10 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 1 mL Lysis Buffer and pour it into a 2 mL microcentrifuge 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 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 200 µL of the 50% suspension (corresponding to 100 µL bed volume) into the batch incubation chamber of the spin column barrel. Close the chamber and spin the resin at 10.000-14.000 x g for 20 sec.

Note: Depending on protein expression levels, adjust the amount of Glutathione Agarose to 200 µl bed volume.

6. Add 600 µL of Wash Buffer and centrifuge again at 10.000-14.000 x g for 20 sec.
7. Repeat the step to completely remove any residual ethanol that might interfere with protein binding to the affinity resin.
8. Immediately before loading, filter the cleared lysate prepared in step 4 through a 0.2 µm filter (e.g. syringe filter) to remove any solid material that might clog the column.

Note: It is critical to perform this filter step immediately before loading the column.

9. Empty the 2 mL centrifuge tube and place the spin column barrel containing the equilibrated purification resin back into it.
10. Load the lysate filtered in step 8. The maximum loading volume is 600 µL. Invert 2-3 times to mix sample and resin. Incubate at 4°C for 1 h on an end-over-end shaker.
11. Centrifuge at at 10.000-14.000 x g for 20 sec, or until the lysate has completely passed through, and collect the flow-through.

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

12. Wash twice with 600 µL each of Wash Buffer.

These are the **wash fractions.**

13. Optional: To remove contaminants such as chaperones, perform an additional wash step with ATP buffer.
14. Replace the 2 mL microcentrifuge tube with a fresh one, and elute the GST-tagged protein by adding 50-600 µL Elution Buffer and centrifuging for 20 sec at 10.000-14.000 x g.

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

15. Repeat step 14 four times, for a total of five elutions. Collect each elution fraction separately.
16. Determine the protein concentration of the elution fractions with Bradford assay, using BSA as protein standard.
17. 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.

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