

Purification of GST-tagged proteins using PureCube Glutathione Agarose and PureCube 1-step batch Midi Plus 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 200 mL IPTG-induced bacterial culture of a well-expressed protein (approximately 10–50 mg/L). The protocol uses the revolutionary 1-step batch Midi Plus Columns that feature the SelfSeal™ membrane technology, saving time and pipetting steps. They replace disposable gravity flow columns used in standard protocols. Volumes from 1 to 20 mL can be applied to a Midi Plus Column. For small scale experiments, 1-step batch Mini 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 Midi Plus 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 Midi Plus Columns (Cube Biotech#63203)
- Ice bath
- Refrigerated centrifuge for 50 mL tube (min. 10,000 x g)
- 50 mL centrifugation tube
- Micropipettor
- Micropipetting tips
- 50 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 #61262)
- Triton X-100
- Reduced Glutathione (e.g. Cube # 61033)
- Optional: Igepal CA-630 (Nonidet P40)
- Optional: Tween-20
- Optional: Adenosine triphosphate (ATP)
- Optional: MgSO₄
- 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 (Nonidet 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 200 mL bacterial culture on ice for 15 min.
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.

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

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 μ L bed volume) into the batch incubation chamber of the spin column barrel. Use the **clear** spin push cap to close the chamber and spin the resin at 400 x g for 5 min.
6. Add 15 mL of Wash Buffer and centrifuge again for 5 min at 400 x g.
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 μ m filter (e.g. syringe filter) to remove any solid material that might clog the column.
9. Empty the 50 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. Tightly screw the **yellow** batch incubation cap and invert 2-3 times to mix sample and resin. Incubate at 4°C for 1 h on an end-over-end shaker.
11. After batch incubation, replace the **yellow** cap with the **clear** spin push cap. Centrifuge at 400 x g for 5 min, or until the lysate has completely passed through, and collect the flow-through.
12. Wash twice with 15 mL each of Wash Buffer.
13. Optional: To remove contaminants such as chaperones, perform an additional wash step with ATP buffer.
14. Replace the 50 mL conical centrifuge tube, and elute the GST-tagged protein by adding 1 mL Elution Buffer.
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.
18. Optional: Perform Western Blot experiment using GST Antibody.

Note: Depending on protein expression levels, adjust the amount of Glutathione Agarose to 250 - 1000 μ L bed volume.

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

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

These are the **wash fractions**.

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

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

