

Expression and Purification of GST-tagged Proteins from *E. coli*

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[Abstract] This protocol describes a method for the small and large-scale expression and purification of GST proteins. Due to the diverse nature of proteins, a small-scale expression and purification test is always recommended.

Materials and Reagents

1. *E. coli* strain of choice
2. IPTG (Sigma-Aldrich)
3. Imidazole (Sigma-Aldrich)
4. Protease inhibitor (Roche Diagnostics)
5. Lysozyme (Sigma-Aldrich)
6. Gluthathion sepharose 4B beads (Thermo Fisher Scientific/Pierce Antibodies, catalog number: 15160)
7. Glutathione (Thermo Fisher Scientific/Pierce Antibodies, catalog number: 78259)
8. NP-40 (Sigma-Aldrich)
9. Bacto trypton
10. Yeast extract
11. Liquid nitrogen
12. NaCl
13. KCl
14. Na₂HPO₄
15. KH₂PO₄
16. Protease inhibitor
17. Lysis buffer (see Recipes)
18. 1x PBS buffer (see Recipes)
19. Elution buffer (see Recipes)
20. 2x YT medium (see Recipes)

Equipment

1. Sonicator
2. Centrifuges
3. Pellete
4. Water bath

Procedure

A. Small scale expression and purification test

1. Grow 5 ml *E. coli* culture harboring the expression construct in 2x YT medium overnight (12-16 h) at 37 °C.
2. The next day, 1:20 dilute the overnight *E. coli* culture into fresh 2x YT medium. Grow at 37 °C to OD₆₀₀ of 0.6-0.8. Depending on *E.coli* strain and status, the time should be around 2-3 h.
3. Induce the culture with 0.1 mM IPTG. Take 10 ml culture out at 0 h, 1 h, 2 h, 4 h and overnight, spin down and flash freeze in liquid nitrogen, then stored in -80 °C.
4. Once all the samples are collected, take the pellete, resuspend in 1.5 ml lysis buffer. Incubate on ice for 30 min.
5. Sonicate each sample on ice for 15 sec for twice.
6. Centrifuge the lysate at top speed at 4 °C for 30 min. Separate the supernatant and pelletes. Resuspend the pelletes in 1.5 ml lysis buffer. Take 10 µl from supernatant and pellete suspension for sampling.
7. Add the 10 L glutathione sepharose 4B beads, incubate at 4 °C for 1 h and wash with 200 µl 1x PBS three times. Collect the supernatant after spinning down the beads for sampling.
8. Elute the beads with 10 µl elution buffer.
9. Run the samples from each step in appropriate SDS-PAGE gel to determine the expression and purification condition, and protein expression amount.

Note: If there is solubility issue, try decrease the IPTG concentration and expression temperature.

B. Large scale expression and purification

1. Grow 50 ml *E.coli* overnight culture at 37 °C.
2. Dilute the over night culture to 1 L culture and grow at 37 °C till OD₆₀₀=0.4-0.8.
3. Induce with 0.1 mM IPTG and grow at 37 °C for appropriate amount of time.

4. Harvest the cells at $4,000 \times g$ for 10 min at 4°C . Flash freeze the pellet in liquid N_2 and store at -80°C .
5. Thaw the cells in room temperature (RT) water bath. Put in ice and resuspend in 5-7 \times pellet volume of lysis buffer supplemented with protease inhibitor and lysozyme (see small scale expression and purification test). Incubate on ice for 30 min.
6. Sonicate on ice for 15 sec for 3 times, until the viscosity decrease. Be careful not let the lysate temperature goes beyond 10°C . Save 10 μl for sample.
7. Centrifuge the lysate at $40,000 \times g$ for 30 min at 4°C . Discard the pellet. Save samples from supernatant and pellets.
8. According to the estimated GST protein amount from small scale expression and purification test and binding capacity of glutathione beads in the manual, add appropriate amount of beads. To ensure purity, the amount of express proteins should be in excess of beads binding capacity.
9. Add 1% NP-40 to reduce the unspecific binding. Incubate at 4°C for 30 min.
10. Transfer the slurry into a disposable column and collect the flow through.
11. Wash the beads with 20X beads volume PBS. Collect the flow through.
12. Add 1X beads volume of elution buffer. Collect the elution.
13. Repeat step 12 for 5 times.
14. After elution, take small amount of beads, add SDS loading buffer for sample.
15. Run the samples collected from lysate, pellet, supernatant, flow through, wash, elute and beads in SDS-PAGE gel.
16. Collect the elution containing your GST-proteins and dialysis against PBS at 4°C for at least 1 h for three times.
17. Flash freeze at liquid nitrogen and store at -80°C .

Recipes

1. 2x YT medium
 - Bacto tryptone 16 g
 - Yeast extract 5 g
 - NaCl 5 g
 - H_2O 800 ml
 - Adjust pH to 7.2
 - Add H_2O to 1 L. Autoclaved.
2. PBS buffer (pH 7.4) (1 L)
 - NaCl 8 g
 - KCl 0.2 g

Na₂HPO₄ 1.44 g

KH₂PO₄ 0.24 g

H₂O 800 ml

Adjust pH to 7.4.

Adjust volume to 1 L with additional distilled H₂O.

Sterilize by autoclaving.

3. Lysis buffer

50 mM NaH₂PO₄ (pH 8.0)

300 mM NaCl

10 mM Imidazole,

1x Protease inhibitor

1 mg/ml lysozyme

4. Elution buffer

20 mM glutathione

75 mM Tris (pH 8.0)

150 mM NaCl

Acknowledgments

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References

1. Pierce Glutathione Agarose Instructions booklet:
<http://www.piercenet.com/instructions/2162248.pdf>.