

Purification of GST-PLC_δ Overview

Already
completed

E. coli transfected with a plasmid containing the GST-PLC_δ fusion protein under an inducible promoter such as IPTG.



Start an overnight starter culture of transformed E. coli



Use the starter culture to seed an expression culture and incubate to an O.D.₆₀₀ of about 0.5.



Induce protein expression with IPTG



Harvest cells by centrifugation



Resuspend cells in PBS buffer



Sonicate cell pellet



Add TritonX-100 and centrifuge



Purify GST-PLC_δ using GST sepharose



Analyze fractions of GST-PLC_δ purification



Quantitate purified GST-PLC_δ



Analyze activity of GST-PLC_δ



Write a report on your results

Purification Protocol (All samples should be kept on ice (4°C) at all times.) Do Not Freeze!

1. Start an overnight starter culture of transformed E. coli

Scrape off a piece of glycerol stock with a pipette tip and add it to 50 mL in an Erlenmeyer flask of LB with 1X ampicillin and incubate shaking overnight at 37°C.

2. Use the starter culture to seed an expression culture and incubate to an O.D.₆₀₀ of about 0.5.

Add the starter culture to 450 mL of LB with 1X ampicillin in an Erlenmeyer flask. Incubate shaking at 37°C.

3. Induce protein expression with IPTG

Incubate until bacterial growth has reached an optical density (O.D.) of about 0.5. Add isopropyl-1-thio-β-D-galactoside (IPTG) to a concentration of 0.1 mM. Continue incubation for 2-4 hours.

Aliquot out 500 mL of culture, centrifuge 1 minute at 10,000 x g, pour off supernatant, label "stage 1", and store in your freezer box.

4. Harvest cells by centrifugation

Transfer bacterial culture to centrifuge tubes and centrifuge 5000 x g for 10 minutes. Pour off the supernatant. Bacterial pellets can be stored at this point at -20°C.

5. Resuspend cells in PBS buffer

Resuspend the bacterial pellet in 25 mL of ice-cold PBS and transfer to a 50 mL conical tube that has the top third of the tube removed. Store on ice.

6. Sonicate cell pellet

Sonicate the bacterial slurry for 2 rounds of 30 seconds at 70% power alternating every other second. Store on ice.

7. Add TritonX-100 and centrifuge

Add 10% (w/v) Triton X-100 to 1% (w/v) final volume and mix. Centrifuge 5 minutes at 10,000 x g at 4°C to remove insoluble material and cells (Short Protocols in Molecular Biology). Transfer supernatant to a 50 mL conical tube. Transfer 0.5 mL of supernatant to a microcentrifuge tube and label it "stage 2". Store on ice.

8. Purify GST-PLC_δ using GST sepharose*

*The following "Batch Method" of the Glutathione Sepharose 4 Fast Flow protocol is taken from the handout from Amersham Pharmacia.

Preparation of Glutathione Sepharose 4 Fast Flow

1. Gently shake the bottle of Glutathione Sepharose 4 Fast Flow to resuspend the slurry.
2. Use a pipette to transfer 1 mL of slurry to a 15 mL conical tube.
3. Sediment the matrix by centrifugation at 500 x g for 5 min. Carefully decant the supernatant.
4. Wash the Glutathione Sepharose 4 Fast Flow by adding 5 mL PBS to the slurry. Invert to mix.
5. Sediment the matrix by centrifugation at 500 x g for 5 min. Carefully decant the supernatant.
6. Repeat steps D. and E. one more time.
7. Resuspend Glutathione Sepharose 4 Fast Flow with 1 mL PBS.

Batch Purification (Be extremely carefully not to lose the matrix during wash steps!)

1. Add the cell lysate to the prepared Glutathione Sepharose 4 Fast Flow and incubate for 20-30 min. at room temperature rocking gently.
2. Sediment the matrix by centrifugation at 500 x g for 5 min. Transfer 0.5 mL to a microcentrifuge tube and label it "flow-through". Carefully decant the supernatant into a new 50 mL conical tube.
3. Wash Glutathione Sepharose 4 Fast Flow by adding 5 mL of PBS. Invert to mix.
4. Sediment the matrix by centrifugation at 500 x g for 5 min. Transfer 0.5 mL to a microcentrifuge tube and label it "wash". Carefully decant the supernatant.

5. Resuspend Glutathione Sepharose 4 Fast Flow with 1 mL of PBS and transfer to a microcentrifuge tube.
6. Sediment the matrix by centrifugation at 500 x g for 1 min. Carefully decant the supernatant.
7. Repeat steps 5 and 6 for a total of three washes.
8. Elute the bound protein by adding 0.5 mL 50 mM Tris•HCl, 10 mM reduced glutathione, pH 8.0. Incubate at room temperature for 5-10 minutes using gentle agitation.
9. Sediment the matrix by centrifugation at 500 x g for 1 min. Carefully transfer the supernatant to a new microcentrifuge tube and label it "elution 1".
10. Repeat steps 8 and 9 and label the second microcentrifuge tube "elution 2".

9. Analyze fractions of GST-PLC δ purification

Run a protein standard, stage 1, stage 2, flow-through, wash, elution 1, and elution 2 on a 12% SDS gel. Determine relative purity of your purified samples by comparing the purified sample lanes to the stage 1 sample.

10. Quantitate purified GST-PLC δ

Conduct a Bradford assay on your eluted samples of purified GST-tagged protein to determine the concentration of protein in your sample. Store on ice.

11. Conduct Western Blot analysis on your GST-tagged protein.

12. Analyze activity of GST-PLC δ

Conduct a PIP-Strip™ analysis of your protein as outlined in the protocol provided by Echelon Biosciences Incorporated.

13. Write a report on your results

Write a report of your experiments in the format of a scientific paper using the Journal Biological Chemistry format.