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GST tagged recombinant protein expression and purification with thrombin cleavage

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We use this protocol and it's working

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Abstract

A protocol to purify GST tagged recombinant proteins from *E. Coli*

Materials

Thrombin (MW 37kDa), Sigma # T-6884: 10 U of thrombin used to cleave 1 mg of fusion protein at 4 degrees overnight.
Glutathione Agarose, Sigma# G4510
1 X Thrombin buffer: 20 mM Tris pH 7.6, 100mM NaCl, 5mM MgCL2,2mM CaCl2, 10% glycerol

Protein expression

1 Transform construct of interest into BL21 GOLD DE3 competent cells.

2 Pick single colonies the next morning and incubate (37C, shaking) in 1ml LB with proper antibiotic as a starter culture.

3 Add the starter culture to 50 ml LB with antibiotic at the end of the day, grow overnight (37C, shaking).

4 The next morning, add the 50ml culture to 2L LB with antibiotic.

5 Incubate the culture for 2-3 hours (37C, shaking), check the absorbance periodically. When the culture reaches 0.6-0.8 at OD600, add IPTG (0.4mM final concentration).

6 Grow for another 3 hours and spin down for 30 min 3000 rcf, 4°C . Store the pellet at -80C.

Protein purification

7 Thaw and resuspend the pellets in thrombin buffer (20 mM Tris pH 7.6, 100mM NaCl, 5mM MgCL2,2mM CaCl2, 10% glycerol) with 1mM DTT and protease inhibitor.

8 Pass the resuspended pellets through an 18G syringe.

9 Mechanically lyse the cells using a cell disruptor. Take care to keep the samples on ice as much as possible.

10 Spin the lysate down for 30 min 20000 rcf, 4°C and save supernatant.

11 Wash GST-agarose beads 2X in thrombin buffer and then add the supernatant to the beads.

12 Incubate the beads for 2 hours at 4C.

13 Wash 3X with buffer (No protease inhibitor).

14 Transfer to 1.5ml tube.

15 Save some beads before cutting GST-tag.

16 Add Thrombin protease (500ul buffer+10ul (10 Unit) Thrombin for 500ul beads) and let it cut overnight in the cold room spinning slowly.

17 Next morning separate supernatant (elution1).

18 Add another 500ul buffer, mix it, separate supernatant (elution2).

19 Span Freeze proteins in small aliquot.

20 Run protein gel for Coomassie stain.

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