Gozani Lab
5/2005

GST-Tagged Protein Expression and Purification

1. Protein Expression (BL21)

Inoculate a starter culture of 10ml in a 250ml flask
Shake overnight at 37˚C
Bring volume of culture up to 125ml and add ZnCl to 50uM final concentration
Shake for 1 hr at 37˚C
Stimulate culture with 0.1 mM IPTG
Shake for 2.5 hrs at 37˚C
Spin 7K 4C for 10 minutes
Pour off waste and either begin lysis or store pellet at –80˚C

For Low Temperature Expression:
Inoculate a starter culture of 10ml in a 250ml flask
Shake overnight at 37˚C
Bring volume of culture up to 125ml and add ZnCl to 50uM final concentration
Shake for 1 hr at 37˚C
Stimulate culture with 0.1 mM IPTG
Shake for 24 hrs at 25˚C
Spin 7K 4C for 10 minutes
Pour off waste and either begin lysis or store pellet at –80˚C

2. GST-Tagged Protein Purification from Bacteria

Buffers:

Lysis Buffer
50 mM Tris pH 7.5
150 mM NaCl
0.05% NP-40

Elution Buffer
50 mM Tris pH 8.0

EB + Glutathione – *Make fresh every time*
6.8 mg Reduced Glutathione (Sigma) per 1 ml Elution Buffer (~ 10 mM)
10 mg/1.5 ml

Procedures:

Resuspend the bacterial pellet from 1 L culture in 40 ml chilled Lysis buffer **(25:1)**
Transfer to a 50ml conical tube and leave on ice for 10 - 20 minutes

Break cells by sonication: Incubate on ice with 0.25 mg/ml lysozeme for 0.5-1 hr; sonicate at output of 18% for 10 sec. (0.5 sec sonication with 1 sec. break) of Branson Digital Sonifier. Repeat once. Save 100-200 ul sample as total lysate (Sample 1)

Transfer to a 15 ml JA20.1 centrifuge Tube, Spin 12K rpm (~18.5 Kg) (or 15 K rpm) at 4˚C for 15 -20 minutes. Save 100-200 ul supernatant as Sample 2.

While the lysed bacteria are spinning prepare the glutathione beads (Amersham)
Using a cut tip P1000 to take about 0.5 ml of the (75%) slurry and transfer to a 15 ml Falcon tube, bring the volume to 5 ml -10 ml with cold lysis buffer, spin the beads at 2.5K rpm of JA 5.3 rotor at 4˚C for 3 minute (or 500g (1500 rpm) for 5 min)

Remove the supernatant and wash the beads at least two more times with lysis buffer.
After the final wash, bring the volume to 0.8 ml and resuspend the beads (to 50% slurry)

After spinning the lysed bacteria, transfer 10 ml supernatant to 15 ml conical tube
To each tube add 200 µl of washed 50% glutathione beads (1 vol. bed of 100 vol. lysate)

Rotate at 4˚C for 90 minutes to 2 hours (can leave for O/N)

Spin 2.5K at 4˚C for 3 minutes (or 500g 5 min)
Remove the supernatant (except for 0.5 – 1ml; and save sample as Sample 3) and wash with 10ml of Lysis Buffer (*rotate 10 min each wash*)

Repeat the spin and remove the supernatant (except for 0.5 – 1ml)

Wash the beads with 10 ml of Elution Buffer
Repeat the spin and remove the supernatant (except for 0.5 – 1ml)

Using a cut tip P1000, resuspend the beads in the remaining supernatant and transfer to a 1.5 ml microfuge tube (combine beads from two tubes if the same sample)

Bring the volume to 1.5 ml with Elution Buffer
Spin 4K at 4˚C for 1 minute at table microcentrifuge.

Let beads settle on ice. Remove supernatant

Resuspend beads in 400-500 µl of EB + Glutathione
Rotate at 4˚C 4 h to overnight

Spin at 500g for 5 min. Remove 350-450 ul supernatant (leave ~30 ul above the beads, avoid pipette in the beads!) and determine protein concentration.

Add DTT to 20 mM final; store eluted protein at 4^{o}C.
Spin at 13K rpm for 10 min before pipette protein for further experiments.

Protein Concentration

Read the OD280 on a 1:50 dilution of the prep.
Multiply this value by 37.5 to obtain the protein concentration in µg/µl
For GST tag, 1 A280=~0.5 mg/ml