Sugden Lab.

Making GST fusion proteins:(07/19/03) ver.1

Grow up 5ml LB with Amp o/n.

Add to 45ml LB with Amp

37° shake 2.5 – 3 hrs, till OD_{600} 0.4-0.8

Put bottles in room temperature water for 10 min to cool down.

Add 100ul 0.2M IPTG to 0.4mM final

Shake 30° 2hr

Pellet bacteria, decant sup, invert to drain

Resuspend in 1ml NETN+0.2mM PMSF / 50ml LB

PMSF, stock 10mM

NETN: 20mM Tris-Cl (pH8)
100mM NaCl
1mM EDTA
0.5% NP40
store at 4°

Vortex to mix well

Sonicate at scale 5 for 15sec. Keep on ice. For 10ml Corning tubes, use scale 7

Spin 4°, 5min

Transfer supernatant to a new tube.

To each lysate, add 60ul 50% Glutathione-Sepharose 4B

Pepette 400 ul Sepharose stock (75%)
Spin 1000rpm 5min, discard supernantant
Wash 3x300ul NETN
Resuspend in 300ul NETN to get 50% beads

Mix in cold room for 2 hours, slowly whirl

Pellet beads by brief centrifugation, carefully discard supernatant

Wash 3x1ml NETN/PMSF

Wash 2x1ml Elution Buffer (50mM Hepes, pH7.9, 40mM KCl, 1mM EDTA 1mM DTT)

Elute proteins by mix beads with 60ul each

Elution buffer
5mM Glutathione, (for 10mM, use 3.07mg/ml)
1mM DTT

Slowly swirl at RT 1hr

Quick spin to pellet, transfer supernatant to a new tube

Re-elute with 60ul each
NETN
5mM Glutathione
1mM DTT

Slowly swirl at RT 30min

Quick spin, combine supernatant, spin and transfer supernatant twice to avoid any residual beads. total is 120ul now.

Dialyze vs 50% glycerol/10mM Hepes, pH7.5/ 40mM KCl/ 1mM EDTA/ 1mM DTT/ 1mM PMSF in cold room for 2hr or o/n, store at -20°

Proteins can also be concentrated in a Centriprep-30 concentrator. The pore size of the membrane in the Centriprep-30 allows glutathione to pass into the aqueous compartment. PBS can be added to the protein concentrate and the concentration procedure can be repeated.

Thinking aliquot and save at -80°

Run 12% SDS-PAGE