Sugden Lab.

GST-fusion protein purification ver.3

Small scale fusion protein preparation

Grow 5ml culture o/n in TB with amp.

Add o/n culture to 50ml of TB+amp and grow for 3 hours in 37ºC shaker.

Induce culture by adding 20µl of 1M IPTG (final [0.4mM]) and transfer to 25ºC shaker for 1hour.

Pellet Bacteria 10min at 3K

Resuspend bacteria in 1ml of NETN+protease inhibitors.

Sonicate 2x for 5-10seconds each time.

Spin out cell debris by spinning in cold microfuge 5 min. at max.

Remove supernatant and store at -70ºC.

Binding fusion protein lysate to beads

Remove 400µl of GST beads into eppendorf and spin 15 sec at 8k.

Remove supernatant and wash 2x with NETN.

Resuspend beads in 320µl of NETN (~550µl final volume)

Aliquot 50µl of beads and add up to 200µl of GST lysate. If < 200µl bring final vol. up to 200µl with NETN.

Incubate at 4ºC with rocking for 30min.

Wash 3x with NETN and add lysate containing protein of interest.

Incubate 1-2hr at 4ºC with rocking.

Wash 3x with appropriate salt buffer.

Boil proteins off in 2xSB for western blotting.

NETN

20mM Tris pH8.0
100mM NaCl
1mM EDTA

2xSB

125mM Tris pH6.8
20% Glycerol
4.1% SDS

Last Modified 07/19/05

0.5% NP-40

2%BME
0.005% Bromphenol Blue

GST Purification Large Scale Protocol

Resuspend 500ml pellet in 10ml of NETN and mix well (Keep at 4ºC)

Combine two pellets (20ml) and transfer 20ml of resuspension to 50ml conical

Sonicate 2x with 15 sec pulses

Spin for 20 at 10k in Sorvall at 4ºC.

Transfer supernatant into new 50ml tubes.

Add 1ml of washed beads rock for 30min at RT

Pour sample into column and drain

Save flow through and repeat previous step 2x

Wash beads with 10ml of 1xPBS 3x

Add 500µl of Elution buffer and rock for 10min at RT

Drain and collect 500µl of fraction

Repeat 2x more

Elution Buffer (5ml)

10mM glutathione	15.6mg
50mM Tris pH 8.0	125µl
H_2O	4.9ml

NETN

20mM Tris pH 8.0
100mM NaCl
1mM EDTA
0.5% NP-40