For GST-tag purification

Cell Lysis

Lysis Buffer
40mM HEPES, pH7.5
0.5 M NaCl
10% glycerol
0.1% NP40
Proteinase Inhibitor

For 10cm plate:

1. Remove medium from the plate.
2. Wash cells with cold PBS. (10ml)
3. Add 5ml lysis buffer to the plate. (or 2-5ml → increase protein conc.)
4. Leave on ice for 10min.
5. Shake the plate and transfer the cell lystate to a 15ml conical tybe.
6. Centrifuge the lystate for 3-5min (2000rpm).
7. Recover the supernatant and store it at –80°C.

GST fusion protein purification: < 1ml

1. Add 7.5 mg glutathoine-agarose for each plate. Rotate at 4°C 60-90 min.
2. Load the mix into a mini-column. Wash with 5X5ml of buffer G plus 0.1%TritonX100 followed by 5X5ml of Buffer G.
3. Elute Gst fusion protein with Buffer G plus 10mM glutathoine(0.031g/10ml), pH8.0 (glutathoine is acidic so check pH of the final solution—need 12µl 10M NaOH to bring 10ml of glutathione solution back to 8.0) in the following manner: resuspend beads in 1ml glutathione solution, incubate at room temperature for 10min, and then elute. Repeat elution 4 more times.

30 mg for 1 ml lysates out 5ml total lyste (1-2 150mm dish 293T

Buffer G
50mM Tris (pH8)
100mM NaCl
0.5mMEDTA
1mMDTT
10% glycerol