

PURIFICATION OF MAMMALIAN GST-BAG1

Materials/Reagents:

PGEX4t-1 Bag-1

IPTG (1M)

TEN100 (Tris / EDTA 0.5mM/NaCl
100mM)

BL21/DE3

LB/amp (100µg/µl)

Protease Inhibitor Tablet

Centri-prep 30

Thrombin

Procedure:

Day 1: Transform BL21/DE3 with pGEX4T-1 Bag-1

Day 2: Restreak one colony from transformation.

Day 3 Pick colony for 25ml overnight starter culture (LB/amp 100µg/µl).

Day 4: Dilute starter culture 40X in LB/amp and grow at until O.D.₅₉₅ ~0.6-0.9.

Induce by adding 1mM final concentration of IPTG, grow for 4-6hrs 30°C.

Harvest by centrifugation 20min. 5,000rpm, 4°C.

Resuspend pellet in 3mls TEN100/gram of pellet.

Add lysozyme (0.5mg/ml) and protease inhibitors (1tablet/20-50mls lysate).

Place on nutator for 30min. 4°C.

Freeze thaw pellet in dry ice methanol bath and 37C water bath (4x).

Sonicate at 30-40%, 3-4 for ~5min. in 30sec. cycles. (cold room)

Clarify by centrifugation 60min., 15,000rpm, 4°C. Take supernatant.

Run through DEAE column on FPLC.

Day 5: Run SDS-PAGE gel of every other fraction to find protein.

Harvest appropriate fractions and run on GST column.

Elute bound protein from column by first washing with 40mls TEN100.

Elute with 10mls elution buffer (9mls TEN100, 1ml glycerol, 0.15 g reduced glutathione). Wash with 40mls TEN100 (collect with 10ml elution).

Cycle remaining protein overnight on GST column.

Day 6: Elute bound protein as before.

Concentrate protein in Centri-prep 30 until ~10-15ml.

Dialyze in 2L TEN100 overnight.

Day 7: Change dialysis buffer.

Day 8: Run protein on Resource Q on FPLC. Run every other fraction on gel

and harvest appropriate fractions. Concentrate with Centri-prep and quantitate.

Flash freeze protein and store as GST-Bag1 or don't freeze and cleave GST.

Incubate protein with thrombin overnight at 22°C to cleave GST.

Day 9: Run over GST column to bind GST and then over benzimidazole column.

to remove thrombin.

Run on gel to check for cleavage; quantitate and flash freeze.

Submitted by: Josh Bosman