GST protein Isolation

KEEP EVERYTHING ON ICE AT ALL TIMES

Day 0 (optional)

- Perform transformation in BL21 cells
- Plate in Lb-agar + antibiotic

Day 1

- Start culture from transformation (Day 0) or glycerol stock

1. Pick large single colony OR
2. Scrape with a pipet tip over frozen glycerol stock

- Place it in 5 ml LB-medium + antibiotic
- Grow for 5-6 hours at 37 degrees in shaking incubator
- Transfer to 250 ml LB + antibiotic
- Incubate o/n 37 degrees

Day 2

- Transfer culture 1:10 to 500 ml LB + antibiotic
- Optional – spin down rest and resuspend pellet in 50% glycerol and store at -80 degrees Celsius
- Incubate 2 hours at 37 degrees Celsius in shaking incubator
- Add 0.3 mM IPTG (Stock is 1 M – so 150 ul in 500 ml culture)
- Incubate o/n at 25 degrees Celsius in shaking incubator

Day 3

- Spin down culture 10 min, 4000 rpm, 4 degrees Celsius (JLA 16-250 – Beckman)

- Resuspend pellet in 10 ml **Lysis buffer + Triton**

- Split suspension in two and sonicate (in 50 ml tubes in beker with ice+bit water)

1. Time 2 minutes amplitude 50
2. Pulls on 7.5 seconds / pulls off 20 seconds

- (if sound changes its ok but always just finish cycle)

- Spin down 10 minutes, 12000 rpm 4 degrees Celsius (JA 30-50 – Beckman)

- Transfer supernatant to 15 ml tube and keep on ice

- For each protein prepare 500 ul of Glutathion sepharose 4B beads for fast flow (wash 3 times with **Lysis buffer + triton**)

- Add 500 ul of beads to each supernatant and incubate for at least 1 hr at 4 degrees rotator

- Spin down, resuspend beads in 1 ml **lysis buffer + triton** and transfer to 1,5 ml ep

- Wash 2x with **lysis buffer + triton** (30 seconds +- 3000 rpm)

- Wash 3x with **lysis buffer – triton** (30 seconds +- 3000 rpm)

- Resuspend beads in 1 ml **lysis buffer WITHOUT triton** and perform protein assay (Pierce)

- Store beads at 4 degrees Celsius

Pierce BCA protein assay (Kit NAME)

1. Mix 50 parts of reagens A with 1 part of reagents B
2. Add 10 ul of standard (Floris), blank, or samples in 96 well flatbottom plate

3. Add 200 ul of mixed reagents (A+B), mix 30 seconds on plate shaker/table
4. Cover plate and incubate 30 minutes at 37 degrees Celsius
5. Read absorbance at 562 nm (Magellan program -> Create/edit method (pick one) ->start measurement -> choose methode -> when done view result -> transport to excel)
6. Possibly also load on SDS-PAGE and check Ponceau staining for protein presence and size

LYSISBUFFER	**LYSISBUFFER**
+ TRITON	**-TRITON**
50 mM Tris, pH 7,4	**50 mM Tris, pH 7,4**
50 mM NaCl	**50 mM NaCl**
5 mM MgCl2	**5 mM MgCl2**
1 mM DTT	**1 mM DTT**
1% Triton X-100	