

Isolation of proteins from inclusion bodies

Expression of recombinant proteins

Materials:

- LB medium
- 40% glucose
- Amp 100 mg/ml
- IPTG 100 mg/ml in PBS

Methods

- inoculate 100 ml LB medium + 1 ml glucose + Amp with 1 fresh colony
- grow overnight
- inoculate 1 l LB medium + 10 ml glucose + Amp , with 1 ml preculture
- grow till OD600 ~ 0.7, take a 1 ml sample
- prepare fresh IPTG, add 1 ml
- incubate 3 hours
- take 1 ml sample for expression control
- cool culture 15-20' on ice
- centrifuge 30' at 2000 g, 4 °C
- pellet can be stored at -80 °C

Expression control

- pellet the 1 ml samples and wash 2x in 1.5 ml cold PBS
- add 100 µl dH₂O and vortex
- add 20 µl 5x SDS-loading and boil 5'
- run 12 µl on a SDS-PAGE gel
- stain with Coomassie

Purification of inclusion bodies

Stock solutions

- lysozym 50 mg/ml in dH₂O (store at -20 C)
- DNase 1 mg/ml in 50% glycerol, 75 mM NaCl (store at -20 °C)
- MgCl₂ 0.5 M in dH₂O
- Tris-Cl 2.5 M pH 8.0
- NaEDTA 0.5 M in 50 mM Tris-Cl, pH 8.0
- NaAzide 30% in PBS

- Na deoxycholate 10% in dH₂O
- Triton X-100 10%
- 5 M NaCl
- DTT 1 M

Buffers

Solution buffer (13 ml) pH 8.0	50 mM Tris-Cl	260 µl stock
	25% sucrose	3.25 g
	1 mM NaEDTA	26 µl stock
	0.1% NaAzide	43.3 µl stock
	10 mM DTT	130 µl stock
lysis buffer (12.5 ml) PH 8.0	50 mM Tris-Cl	250 µl stock
	1% Triton X-100	1.25 ml stock
	1% Na deoxycholate	1.25 ml stock
	100 mM NaCl	250 µl stock
	0.1% NaAzide	42 µl stock
	10 mM DTT	125 µl stock
washing buffer with Triton (10 ml) pH 8.0	50 mM Tris-Cl	200 µl stock
	0.5% Triton X-100	0.5 ml stock
	100 mM NaCl	200 µl stock
	1 mM NaEDTA	20 µl stock
	0.1% NaAzide	33.3 µl stock
	1 mM DTT	10 µl stock
washing buffer without Triton (10 ml) pH 8.0	50 mM Tris-Cl	200 µl stock
	100 mM NaCl	200 µl stock
	1 mM NaEDTA	20 µl stock
	0.1% NaAzide	33.3 µl stock
	1 mM DTT	10 µl stock

- resuspend pellet in 13 ml solution buffer on ice and transfer it to a 30 ml centrifugion bottle
- sonicate 50% level 4-5, 30 pulses, on ice
- add 100 µl lysozym, 250 µl DNase I, 50 µl MgCl₂
- vortex
- add 12.5 ml lysis buffer and vortex short

- incubate 30-60' at RT
- add 350 µl NaEDTA
- freeze in N2 until it stops bubbling and thaw for 30' at 37 °C
- add 200 µl MgCl2
- wait until viscosity decreases 30-60'
- add 350 ml NaEDTA

from now on everything should be done on ice

- pellet at 11,000 g, 20', 4 °C
- discard supernatant and take a sample
- resuspend pellet in 10 ml washing buffer with Triton
- sonicate 50% level 4-5, 30 pulses, on ice
- pellet at 11,000 g, 20', 4 °C
- discard supernatant and take a sample
- resuspend pellet in 10 ml washing buffer without Triton
- sonicate 50 % level 4-5, 30 pulses, on ice
- pellet at 11,000 g, 20', 4 °C
- discard supernatant and take a sample

Dissolving of recombinant protein

- dissolve pellet in 9 ml 8 M Guanidinium pH 8.0 + 4 mM DTT
- shake at RT until pellet dissolves
- aliquot in 1 ml fractions and store at -80 °C

Refolding

* 200 ml refolding buffer	100 mM Tris-Cl	8 ml stock
	400 mM L-Arginine	16.9 g
	2 mM NaEDTA	0.8 ml stock
	0.5 mM ox.glutathione	61.2 mg
	5 mM red. glutathione	308 mg
	protease inhibitors	50 µl cocktail

- add 1 ml dissolved protein drop by drop using a 1 ml syringe and a # 27 meedle to the refolding buffer while vigorously stirring
- stir slow, 8 hours at 4 °C
- again add 1 ml
- stir slow, 8 hours at 4 °C

- again add 1 ml
- stir slow, 8 hours at 4 °C
- filter through a 2 um filter and concentrate till ~ 12 ml in a 200 ml concentrator
- wash concentrator with 1 ml flow through and add this to the protein sample
- measure OD280, it should be at least 3x higher than flow through

Size exclusion(FPLC)

Buffer

* elution buffer 150 mM NaCl
 20 mM Tris-Cl pH 8.0 (2um filtered)

- filter the protein sample through a 0.2 um filter
- bring 13 ml protein on the size exclusion column (hiload 26/60 Superdex 200 prep grade) using a superloop
- elute with the elution buffer 1ml/min, start collection 4 ml fractions from 70 ml-300 ml (unfolded protein comes of in the void volume)
- determine amount of protein in different fractions and run fractions on gel to check the purity of the protein