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IPTG-induced Overexpression in E. coli

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1 Works for me dx.doi.org/10.17504/protocols.io.762hrge

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ABSTRACT

- LB
- 1M IPTG
- BPER
- HEPES

Protein Expression (optimised for inclusion bodies)

Take volume (100ml) LB+ Ampicillin
Add 1 ml of the over night culture
Let the culture grw to an OD of 0.6 at 37°/180 rpm
Induce with 0,5 mM IPTG (1M Stock) --> 50 µl
Incubate for 2 hours at 37°/ 180 rpm
Centrifuge the culture for 10 minutes at 4000xg / 4°C
Store Pellet without supernatant at -80°

Purification of inclusion bodies

Thaw cell pellet on ice
Resuspend Pellet in 1 ml Water (2x50ml Pellets from 100ml culture can be resuspended together)
Transfer to 2 ml Eppis
Centrifuge at maximum speed of a desktop centrifuge at 4°C for 15 minutes
Discard supernatant
Resuspend Pellet in 0,5 ml BPER by vortexting
Repeat vortexing every 2 minutes for 15 minutes
Centrifuge at maximum speed of a desktop centrifuge at 4°C for 15 minutes
Store the supernatant (BPER) in seperate Eppis (on ice)
Dilute 1 ml BPER with 9 ml Water to create 1/10 BPER
Wash the pellet with 1 ml 1/10 BPER (can be done by vortexing for 3x1 minute)
Centrifuge at maximum speed of a desktop centrifuge at 4°C for 15 minutes
Store the supernatant (1/10 BPER) in seperate Eppis (on ice)
Resuspend Pellet in 200 µl 50 mM HEPES pH7.4, 150 mM KCl, 10% Glycerol (If you do not intend to use your protein for activity assays, Water can also be used)
Put samples on SDS-Gel or store at -20°C. On an SDS-Gel mix 0,5-1µl of sample + 4 µl 5xSDS-Buffer and put on the gel