

Oct 14, 2019

IPTG-induced Overexpression in E. coli

DOI

dx.doi.org/10.17504/protocols.io.762hrge

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DOI: dx.doi.org/10.17504/protocols.io.762hrge

Protocol Citation: Igem Dusseldorf 2019. IPTG-induced Overexpression in E. coli. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.762hrge>

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Protocol status: Working

We use this protocol and it's working

Created: October 14, 2019

Last Modified: October 14, 2019

Protocol Integer ID: 28602

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 grow 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 (2×50ml 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 vortexing

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 separate 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 3×1 minute)

Centrifuge at maximum speed of a desktop centrifuge at 4°C for 15 minutes

Store the supernatant (1/10 BPER) in separate 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

