IPTG-induced Overexpression in E. coli

iGEM Dusseldorf[1]

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1 Works for me

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MATERIALS TEXT

- LB
- 1M IPTG
- BPER
- HEPES
- ICE
- 150 mM KCl
- 10% GLYCEROL
- WATER
- 5xSDS-Buffer

Protein Expression (optimised for inclusion bodies)

1 Protein Expression (optimised for inclusion bodies)

1. Take volume (100 ml) LB+ampicillin
2. Add 1 ml of the overnight culture
3. Let the culture grow to an OD_{600} of 0.6 at 37°C/180 rpm
4. Induce with 0.5 mM IPTG (1M Stock) --> 50 µl
5. Incubate for 2 hours at 37°C/ 180 rpm
6. Centrifuge the culture for 10 min at 4000xg / 4°C
7. Store cell pellet without supernatant at -80°C

2 Purification of inclusion bodies

1. Thaw cell pellet on ice
2. Resuspend pellet in 1 ml water (2x50 ml pellets from 100 ml culture can be resuspended together)
3. Transfer to 2 ml reaction tubes
4. Centrifuge at maximum speed of a desktop centrifuge at 4°C for 15 minutes
5. Discard supernatant
6. Resuspend pellet in 0.5 ml BPER by vortexting
7. Repeat vortexing every 2 minutes for 15 minutes
8. Centrifuge at maximum speed of a desktop centrifuge at 4°C for 15 minutes
9. Store the supernatant (BPER) in seperate reaction tubes (on ice)
10. Dilute 1 ml BPER with 9 ml water to create 1/10 BPER
11. Wash the pellet with 1 ml 1/10 BPER (can be done by vortexing for 3x1 minute)
12. Centrifuge at maximum speed of a desktop centrifuge at 4°C for 15 minutes
13. Store the supernatant (1/10 BPER) in seperate reaction tubes (on ice)
14. Resuspend pellet in 200 µl 50 mM HEPES pH 7.4, 150 mM KCl, 10% Glycerol (If you do not intend to use your protein for activity assays, water can also be used)
15. Put samples on SDS gel or store at -20°C. On an SDS gel mix 0.5-1 µl of sample + 4 µl 5x SDS buffer and put on the gel.