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## Protein expression using *E. coli* strain BL21DE3

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**Protocol status:** Working

**We use this protocol and it's working**

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### Abstract

In this protocol will be explained how to express proteins using *E. coli* strain BL21DE3 using a plasmid with a T7 promoter. The promoter of T7 polymerase from *E. coli* BL21DE3 has to be induced with IPTG.





## Day 1

- 1 Prepare an overnight culture of the bacterium encoding for the protein you want to express.

16h

## Day 2

- 2 Prepare an Erlenmeyer flask containing LB medium and the right antibiotics
- 3 Inoculate the Erlenmeyer flask 1:100 from your overnight culture and grow the bacteria at 37°C and 120 rpm.
- 4 Grow the culture to an OD600 of 0.6. This will usually take 2-3 hours.
- 5 Take 1 ml of pre-induction sample and cold shock the culture for 30 minutes on ice. Keep the pre-induction sample in the fridge or freezer.
- 6 Add IPTG to a final maximum concentration of 1μM. IPTG is used because *E. coli* BL21DE3 has its T7 polymerase gene under an IPTG sensitive promoter and we have used plasmids with a T7 promoter in our protein expression experiments
- 7 Grow the culture overnight (for 18hours) at 20°C and 120 rpm.

3h

30m

18h

## Day 3

- 8 Measure the OD600 of a 10x diluted post-induction sample. Calculate the "real" optical density by multiplying this number by 10.
- 9 Take 1 ml of post-induction sample and spin this, together with the pre-induction sample down for 10 minutes at 4700 g, using a tabletop centrifuge.
- 10 Resuspend the pellet of the pre-induction sample in 100μl of MQ. To correct for the difference in OD600 when you load the samples on a protein gel, resuspend the pellet of the post-induction sample in 100μl x  
("real"OD600 post-induction sample)/(OD600pre-induction sample)

10m



- 11 Prepare the samples to be loaded on a protein gel
- 12 Run both the pre-induction and post-induction sample on a protein gel