Condensed protocol for Protein Expression: Step-by-step Procedure

Last updated: 2026-08-12

Reviewed by: Rytis

Notes: This protocol is for the expression of atcABC cloned in pET21 (from TwistBioscience) and moaABCDE_mobAB cloned in pCDFDuet1 (from SynBio) and sampling for subsequent SDS-PAGE, purification and quantification.

Materials

- Host strain: *E. coli* BL21(DE3), transformed with vector containing gene of interest.
- Media: TB
- Antibiotic: Ampicillin (final concentration 100 µg/mL), spectinomycin (final concentration 100 µg/mL)
- IPTG stock: 100 mM (filter sterilized)
- **sodium molybdate (Na_2MoO_4) stock aqueous solution: 100 mM** (filter sterilized or autoclaved)
- Iron citrate (or $FeCl_3$) stock: 10 mM (filter sterilized or autoclaved)

  Equipment:

  - Shaking incubators*2 (37 °C and 18°C, 150–170 rpm)
  - Spectrophotometer (OD_{600})
  - Cuvettes + sterile H_2O
  - Shake flasks
  - Consumables: Sterile pipettes, tubes, SDS-PAGE sampling supplies.

Procedure

1. Place the overnight culture on ice.
2. Pool together atcABC+moaABCDE_mobAB culture tubes using a pipette in one tube, keep it on ice
3. Measure OD600 of the starter cultures, you will likely have to dilute them 10x in LB Medium in the cuvette
4. Inoculation volume needed: for 100 mL media (main culture) inoculate 0.5 mL of starter culture with OD 600=3, final OD~0.01-0.02
5. Mix Solution A and B (for TB media)
6. Add 100 µg/ml ampicillin + spectinomycin to each individual flask
7. Inoculate flasks
8. Incubate at the chosen temperature and 170 rpm
    a. Predicted timepoint based on previous measurements should be 3h
    b. Follow the culture, be aware you will need to dilute (10x) normally works ok, you can use TB medium to dilute it
    c. Induction should be done before OD600 actually reaches 1.5
9. Add
    a. **sodium molybdate (Na_2MoO_4) stock aqueous solution to the culture media, to reach 1 mM conc.**
    b. **100 µM Iron(III) citrate (or $FeCl_3$ or Ammonium iron(III) citrate)**
10. OD600 reaches ~ 1.5, this will be T_0 (uninduced, time of induction)
    a. Take the flask out of the incubator
    b. Take 1 sample for OD measurements
    c. Take 1 sample for SDS-PAGE analysis
11. Add IPTG at the chosen concentration and keep incubating the culture at chosen temperature (18ºC, overnight; 28ºC, 3-4h)
12. Take samples for SDS-PAGE and OD600 measurements after 3h/4h
13. Stop the culture

Sampling: Step-by-step Procedure

You will need to recover two different samples: one for OD measurements and one for SDS-PAGE Analysis

For OD measurements:

1. First part of the culture should be enough 900 µL and 100 µL main culture (dilution 10x)
2. Second part of the culture you will probably need 950 µl and 50µl main culture

You can prepare in advance the cuvettes with , cut already pieces of parafilm that you will use to cover the cuvettes when mixing/inverting them before measuring.

SAMPLING:

1. Prepare and label 1.5 mL tubes for SDS samples
2. Take out flasks from the incubator
3. Take the sample (by tilting the flask and not touching the walls by pipette)
4. Put the cultures back into the incubator
5. Measure the OD

After purification, measure absorbance at 415 nm with a spectrophotometer. If it detects, molybdenum is inserted, if not, it is not inserted.