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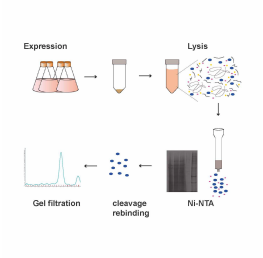
Expression and purification of bacterial proteins (via N-terminal His-tag)



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We use this protocol and it's working

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Abstract

Recombinant protein production in bacteria provides an efficient system for obtaining high yields in a short time and at low cost. Although not every protein is suitable for expression in bacteria, it is usually the first choice to start expression trials. If successful, more elaborate protein purification protocols can be developed. Here we provide a two-step protocol for protein purification from bacterial cells.

Materials

Transformation and protein expression
Chemically competent *E.coli* Rosetta cells
Plasmids encoding protein of interest

LB medium (lysogeny broth) - comercially available
LB agar plates (LB medium + 1% agar)
TB medium (terrific broth) - comercially available
Antibiotics: ampicilin, kanamycin, chloramphenicol
0.5 M Isopropyl-β-d-thiogalactopyranosid (IPTG)

Protein purification
Ni-NTA sepharose - comercially available
Lysis buffer (50 mM Hepes pH7.4; 500 mM NaCl; 20 mM imidazole; 0.5 mM TCEP; 5 % glycerol)
Elution buffer (50 mM Hepes pH7.4; 500 mM NaCl; 300 mM imidazole; 0.5 mM TCEP; 5 % glycerol)

S200 Superdex gel filtration column
Size-exclusion buffer (20 mM Hepes pH 7.4; 150 mM NaCl; 0.5 mM TCEP; 5% glycerol)
Liquid nitrogen

Safety warnings

This protocol requires handling of genetically modified organisms which requires an S1 facility.

Plamid Transformation into *E.coli* Rosetta cells

1d 2h

1 Thaw 10 µL *E.coli* Rosetta cells gently on ice — 20m

2 Add 25 ng respective expression plasmid and keep On ice for 00:30:00 min — 30m
♦ DARPin E11 in pQE30 vector (Qiagen) - this encodes: N-terminal 8xHis-tag and a C-terminal 3xFLAG-tag
♦ Rab8 in pET28A vector (Novagen) - this encodes a N-terminal TEV-cleavable 6xHis-tag

3 Apply heatshock for 00:00:45 sec in a 42 °C waterbath — 45s

4 Let cells recover for 00:05:00 min On ice — 5m

5 Add 100 µL LB medium and incubate cells for 00:30:00 min at 37 °C — 30m

6 Plate cell suspension on LB agar plates containing appropriate selection pressure

7 Incubate Overnight at 37 °C — 5m

8 Plates can be stored at 4 °C for several weeks or until further use

Large Scale Protein Expression (4L)

1d

9 From a single colony of the LB plates inoculate 50 mL LB medium containing the appropriate selection pressure — 10m

10 Incubate the liquid culture shaking at 180 rpm, 37°C overnight — 20h

11 Next morning, dilute grown overnight culture 10 mL into 1000 mL terrific broth expression medium containing the appropriate antibiotic — 1h

12 Grow cells shaking at 180 rpm, 37°C until OD600 reached 1.0 — 4h

> **Note**
>
> This may take about 04:00:00 h but regularly check OD600.

13 Once OD600 reaches 1.0, reduce temperature to 18 °C and wait for another 00:30:00 min before adding [M] 500 millimolar (mM) IPTG — 30m

14 Incubate for 18:00:00 hours shaking at 180 rpm, 18°C — 18h

15 Harvest cells by centrigufation with 1000 x g, 4°C, 00:15:00 min — 25m

16 Freeze pellet and store -20 °C until further use

2-Step Protein Purification — 8h

17 Thaw pellet and resuspend in 300 mL lysis buffer — 20m

> **Note**
>
> Lysis buffer contains
> [M] 50 millimolar (mM) HEPES pH 7.4,
> [M] 500 millimolar (mM) NaCl
> [M] 20 millimolar (mM) imidazole
> [M] 0.5 millimolar (mM) TCEP
> [M] 5 % (v/v) glycerol

18 Lyse by sonication 3 rounds with 5 sec pulse, 10 sec pause, 3 min total pulse time On ice — 30m

19 Clear lysate by ultracentrifugation 1000000 x g, 4°C, 00:45:00 — 1h

20 Perform Ni-NTA 1h

20.1 Load supernatant onto pre-equilibrated Ni-NTA sepharose beads 20m

20.2 Wash beads rigorously with lysis buffer (50 CV) 20m

20.3 Elute protein using lysis buffer supplemented with [M] 300 millimolar (mM) imidazole 20m

21 Concentrate eluate to volume of 5 mL and subject to size-exclusion chromatography on a S200 gel filtration column 3h

Note

size-exclusion buffer contains
[M] 20 millimolar (mM) HEPES pH 7.4
[M] 150 millimolar (mM) NaCl
[M] 0.5 millimolar (mM) TCEP
[M] 5 % (v/v) glycerol

22 Pool fractions containing your protein of interest 30m

23 Measure protein concentration and do additional quality control measurements such as SDS PAGE with Coomassie staining, mass spectrometry to validate you sample 30m

24 Flash freeze aliquots of your protein of interest and store at -80 °C