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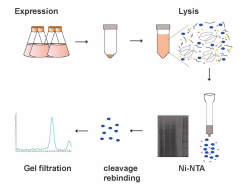
Expression and purification of bacterial proteins (via N-terminal His-tag)

 [Science Advances](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.261ge514yg47/v1>Verena Dederer^{1,2,3}, Sebastian Mathea^{1,2,3}, Stefan Knapp^{1,2,3}¹Institute of Pharmaceutical Chemistry, Goethe University Frankfurt, Max-von-Laue-Straße 9, Frankfurt 60438, Germany;²Structural Genomics Consortium, Buchman Institute for Molecular Life Science (BMLS), Max-von-Laue-Straße 15, Frankfurt 60438, Germany;³Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, USA**Verena Dederer**

Goethe University Frankfurt



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Protocol status: Working

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) - commercially available

LB agar plates (LB medium + 1% agar)

TB medium (terrific broth) - commercially available

Antibiotics: ampicilin, kanamycin, chloramphenicol

0.5 M Isopropyl- β -d-thiogalactopyranosid (IPTG)

Protein purification

Ni-NTA sepharose - commercially 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 500 millimolar (mM) IPTG

30m

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

18h

15 Harvest cells by centrifugation 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

50 millimolar (mM) HEPES 7.4 ,

500 millimolar (mM) NaCl

20 millimolar (mM) imidazole

0.5 millimolar (mM) TCEP

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  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