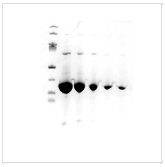

Jul 05, 2021

Purification of recombinant *Synechocystis* KaiA3-His6 with PureProteome Nickel Magnetic Beads

Christin Köbler[1], Nicolas M Schmelling[2], Alice Pawlowski[2], Philipp Spät[3], Nina Scheurer[1], Lutz LCB Berwanger[2], Ilka Axmann[2], Annegret Wilde[1]

[1]Institute of Biology III, Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany;
[2]Institute for Synthetic Microbiology, Biology Department, Heinrich Heine University Duesseldorf, 40225 Duesseldorf, Germany;
[3]Department of Quantitative Proteomics, Interfaculty Institute for Cell Biology, Eberhard Karls University Tuebingen, 72076 Tuebingen, Germany

 dx.doi.org/10.17504/protocols.io.bu5bny2n

Axmann Lab

Alice Pawlowski

ABSTRACT

This protocol can be used to
- express his-tagged proteins in *E. coli* cells by using a T7-polymerase based expression system
- Lyse cells with sonification
- purify target protein by Ni-affinity chromatography with nickel magnetic beads

DOI

dx.doi.org/10.17504/protocols.io.bu5bny2n

PROTOCOL CITATION

Christin Köbler, Nicolas M Schmelling, Alice Pawlowski, Philipp Spät, Nina Scheurer, Lutz LCB Berwanger, Ilka Axmann, Annegret Wilde 2021. Purification of recombinant Synechocystis KaiA3-His6 with PureProteome Nickel Magnetic Beads. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bu5bny2n

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

A chimeric KaiA-like regulator extends the nonstandard KaiB3-KaiC3 clock system in bacteria

KEYWORDS

Ni affinity purification, Ni magnetic beads, KaiA3, Synechocystis, heterologous protein expression, his-tag

CREATED

May 18, 2021

LAST MODIFIED

Jul 05, 2021

PROTOCOL INTEGER ID

50051

MATERIALS TEXT

Reagents

Benzonase nuclease **Sigma Aldrich Catalog #E1014-5KU**

cOmplete™, Mini, EDTA-free (Protease Inhibitor) **Roche Catalog ##11836170001)**

Triton X-100 **Sigma Aldrich Catalog #T8787-50ML**

Imidazole **Sigma Catalog #I5513**

Ampicillin sodium salt **Millipore Sigma Catalog #A0166**

LB Broth (Lennox) vegetal **Carl Roth Catalog #0155**

IPTG **Thermo Fisher Scientific Catalog #R0392**

LB Lennox agar 500 g **Carl Roth Catalog # X965.1**

Dithiothreitol (DTT) **Contributed by users**

ROTI®Blue quick **Carl Roth Catalog #4829.2**

SDS **Millipore Sigma Catalog #436143**

Lysozyme >35000 FIP U/mg **CarlRoth Catalog #8259.2**

Bromophenol Blue sodium salt **Sigma Aldrich Catalog #B5525-10G**

EDTA Disodium Salt **Contributed by users Catalog #PubChem CID: 8759**

Disodium hydrogenphosphate dihydrate, MW: 177.99 **Carl Roth Catalog #4984.1**

Sodium Dihydrogenphosphate monohydrate, MW: 137.99 **Panreac AppliChem Catalog #A3559,1000**

PureProteome Nickel Magnetic Beads **Sigma Aldrich Catalog #LSKMAGH02**

Tuner(DE3) competent cells Novagen **Sigma Aldrich Catalog #70623**

PageRuler Prestained Protein Ladder, 10 to 180 kDa **Thermo Fisher Scientific**

Buffers

0.5 M EDTA (pH 8.0)

186.1 g of disodium EDTA dihydrate ($Na_2EDTA \times 2\ H_2O$)

800 mL of millipore water

Adjust the pH to 8.0 with NaOH (~20g NaOH)

Bring volume to 1 L with dH2O

Stir vigorously on a magnetic stirrer*

Sterilize by autoclaving

Store at room temperature

*The disodium salt of EDTA will not dissolve until the pH of the solution is adjusted to 8.0 by the addition of NaOH

Citation: Christin KÃÂ¶bler, Nicolas M Schmelling, Alice Pawlowski, Philipp SpÃÂ¤t, Nina Scheurer, Lutz LCB Berwanger, Ilka Axmann, Annegret Wilde (07/05/2021). Purification of recombinant Synechocystis KaiA3-His6 with PureProteome Nickel Magnetic Beads. https://dx.doi.org/10.17504/protocols.io.bu5bny2n

0.5 M sodium phosphate stock solution, pH 8.0
prepare 1 M stock solution of Na_2HPO_4 and 1 M stock solution of NaH_2PO_4
use 473.5 ml of the 1 M Na_2HPO_4 solution and add NaH_2PO_4 to pH8.0 (supposed to be 26.5 ml)
adjust to 1 Liter with millipore water

5 M NaCl stock solution
292.2 g NaCl (58.44 g/mol)
ad 1000 ml millipore water

1 M Imidazole (IMZ)
34.04 g IMZ
ad 500 ml millipore water

Lysis buffer
50 mM sodium phosphate buffer pH8.0
300 mM NaCl

Lysis buffer + IMZ, washing buffer
50 mM sodium phosphatebuffer, pH8.0
300 mM NaCl
10 mM IMZ

Elutionbuffer
50 mM sodium phosphate buffer, pH8.0
300 mM NaCl
300 mM IMZ

Storage buffer for magnetic beads
20% Ethanol, freshly prepared

KaiA3 storage buffer
20 mM Tris-HCl, pH 8.0
150 mM NaCl
0.5 mM EDTA, pH 8.0
5 mM $MgCl_2$
1 mM ATP

5 x sample buffer (SB) for SDS-PAGE
250 mM Tris-HCl, pH 6.8
40 % (v/v) glycerol
8 % (w/v) SDS
100 mM DTT
0.1 % (w/v) bromphenol blue

Equipment

Photometer (NanoPhotometer®C40, Implen)
Ultrasonic homogenizer (Bandelin Sonopuls HD 2070) with sonotrode tip KE76
PureProteome magnetic stand, 15 ml (Millipore)
Rotator (Intelli-mixer RM-2)

Citation: Christin KÃÂ¶bler, Nicolas M Schmelling, Alice Pawlowski, Philipp SpÃÂ¤t, Nina Scheurer, Lutz LCB Berwanger, Ilka Axmann, Annegret Wilde (07/05/2021). Purification of recombinant Synechocystis KaiA3-His6 with PureProteome Nickel Magnetic Beads. https://dx.doi.org/10.17504/protocols.io.bu5bny2n

Heterologous protein expression in *E. coli*

1 Transformation

- transform the plasmid pET22*sll0485*_his6 (for expression of the KaiA3-His6 fusion protein) into *E. coli* Tuner(DE3) cells by either using chemical competent cells or by using the TSS transformation method (dx.doi.org/10.17504/protocols.io.gtabwie) and plate on LB agar plates containing ampicillin (100 µg/ml)
- re-streak the transformed cells on fresh agar plates one more time before expression
- prepare a cryo culture (20% glycerol) for storage at -80°C or proceed to step 2

2

- streak Tuner(DE3) cells containing plasmid pET*sll0485*-his6 from the glycerol stock on a LB+Amp agar plate, incubate o/n at 37 °C
- take some colonies to inoculate 20 ml pre-culture in LB-Amp (100 ml Erlenmeyer), incubate o/n at 37 C°, 250 rpm
  NOTE: always use freshly streaked cells (max one week old for KaiA3) for the pre-culture
- inoculate 0.8 liter LB-Amp (4 l Erlenmeyer) with 1/100 volume of the pre-culture and measure the starting OD600 (the starting OD600 is around 0.06 in the Implen photometer)
- incubate culture at 37 °C, 250 rpm until OD600 reaches 0.5 -0.7 (~ 2.5 h)
- induce the expression with 0.5 mM IPTG (= timepoint T0)

→ optional: immediately take a sample for SDS-PAGE (total protein at T0): 200 µl cells, centrifuge, remove supernatant and resuspend pellet in 20 µl H_2O + 5 µl 5x SB, store at -20°C

- incubate for 3.5 h at 37 °C and 250 rpm (or o/n at 28°C)

→ optional: take a sample for SDS-PAGE (total protein at T3.5): 50 µl cells, centrifuge, remove supernatant and resuspend pellet in 20 µl H_2O + 5 µl 5x SB, store at -20°C

- centrifuge cells for 10 min at 4000 x g and 4 °C
- transfer the pellet to a 50 ml falcon tube with a spatula; suspend remaining cell material in lysisbuffer (50mm sodium phosphate buffer pH8, 300 mM NaCl) and transfer the suspension to the falcon tube
- suspend cells completely by vortexing, add more lysis-buffer if necessary
- centrifuge cells for 10 min at 4000 x g and 4 °C
- remove the supernatant, weigh the pellet and store the cells at – 80°C or proceed to next section

preparation of the cell lysate

3

- suspend the cell pellet 1:4 (e.g. 20 ml buffer for 5 g pellet) in lysis buffer + 10 mM IMZ (including protease inhibitor cOmplete tabs Mini without EDTA, lysozyme and benzonase), prepare buffer as follows: 1 tablet protease inhibitor per 10 ml buffer; lysozyme (50 mg/ml) to a final concentration of 0.1 mg/ml (do not exceed 0.1 mg/ml to prevent binding of lysozyme to Ni-Affinity resin); 0.5 µl Benzonase (250 u)
- incubate 30’ on ice with shaking
- disrupt cells with sonification (Bandelin homogenizer Sonopuls):
  6', KE 76 Sonotrode, 60% output, cycle 3 (keep cells on ice during disruption!)
- transfer suspension to a small beaker with a magnetic stirrer and add triton X-100 to a final concentration of 1%
- incubate for 20’ on ice with stirring
- transfer the cell suspension to a 50 mL falcon (check that the tube stands 11000 rpm centrifugation force)
- centrifuge 30’, 11000 rpm, 4°C (meanwhile equilibrate the magnetic beads, see step 4)
- transfer the supernatant to a fresh tube (= soluble fraction)

→ optional: take a sample for SDS-PAGE (supernatant = soluble protein): 5 µl lysate + 15 µl H_2O + 5 µl 5x SB, store at -20°C
→ optional: take a sample for SDS-PAGE (pellet = insoluble protein): suspend the pellet from the sonicated crude extract in the same volume of water as you have lysate; take 5 µl + 15 µl H_2O + 5 µl 5x SB, at -20°C

Ni affinity chromatography

4 equilibration of Ni-magnetic beads

- use the 15 ml PureProteome magnetic stand; the stand fits 2×15 ml falcon tubes; up to 750 µl Ni-magnetic beads can be used (200 µl bead-suspension can bind 200-1100 µg of his-tagged protein)

Citation: Christin KÃÂ¶bler, Nicolas M Schmelling, Alice Pawlowski, Philipp SpÃÂ¤t, Nina Scheurer, Lutz LCB Berwanger, Ilka Axmann, Annegret Wilde (07/05/2021). Purification of recombinant Synechocystis KaiA3-His6 with PureProteome Nickel Magnetic Beads. https://dx.doi.org/10.17504/protocols.io.bu5bny2n

- suspend fresh PureProteome magnetic bead solution (or regenerated beads from the same protein) with vortexing and shaking until you have a homogenous solution
- add 750 µl bead suspension in each 15 ml tube and place the tubes in the magnetic stand
- Remove the storage solution after beads have migrated to the magnet using a 5 ml pipette
- Equilibrate beads in 5 ml lysisbuffer + IMZ (50 mM Na-Phosphatebuffer, pH8, 300 mM NaCl + 10 mM IMZ) by gentle hand-mixing for 1 minute at RT
- Place the tubes into the stand to collect the beads and carefully remove the buffer with a pipette

Binding and washing:

- Add the soluble protein fraction from step 3 to the beads and incubate the tubes with gentle mixing for 30' at RT (intelli-mixer, program F1, 10 rpm)
- Place the tubes back in the stand and let the beads migrate to the magnet; shortly invert the stand with the tubes to remove residual beads from the caps, capture the beads and remove the lysate (= fraction flow through, FT)

→ optional: take a sample for SDS-PAGE (flow through): 5 µl FT + 15 µl H_2O + 5 µl 5x SB, store at -20°C

- Wash the beads by incubating in 5 ml wash-buffer (lysisbuffer with 10 mM IMZ; up to 20 mM IMZ can be used in washing buffer, depending on the protein) with gentle hand-shaking for 1 min at RT
- Place the tubes back in the stand, collect the beads and remove the buffer carefully with a pipette (fraction wash 1)
- Repeat the washing step 2 more times (wash 2+3)

→ optional: take samples for SDS-PAGE (wash): 20 µl W + 10 µl H_2O + 5 µl 5x SB, store at -20°C

Elution of his-tagged protein

- Elute the bound protein by adding 1 ml elution buffer (50 mM Na-phosphate buffer, pH8, 300 mM NaCl, 300 mM IMZ), incubate with gentle mixing for 2 min at RT (intelli-mixer, F1, 10 rpm)
- Place the tubes back in the stand, allow beads to migrate to the magnet and collect eluate carefully into a clean 2 ml Eppendorf-tube (fraction E1)
- Repeat elution one more time (fraction E2)
- Eluates can be stored at 4 °C for 1 – 3 days, for buffer exchange and longterm storage proceed to step 5

→ take samples for SDS-PAGE (eluates): 20 µl E1, E2 + 10 µl H_2O + 5 µl 5x SB, store at -20°C

Regeneration of magnetic beads

5
- incubate the beads in 5 ml a. dest. for 5 min at RT (intellimixer F1, 10 rpm), place in stand, collect the beads and remove the liquid
- repeat washing with a. dest. one more time
- Suspend beads in 5 ml 20 % Ethanol (freshly prepared) and incubate with gentle shaking for 5 min at RT, place back in stand, collect beads and remove ethanol
- Repeat one more time
- Suspend beads in 750 µl 20% Ethanol storage solution (= volume of initially taken bead solution) and transfer to a clean 2 ml Eppendorf-tube; label tubes with name of protein and how often beads have been used; re-use beads for purification of the same protein only
- Store used beads at 4°C

buffer exchange and protein storage

6
- Eluates from Ni-affinity chromatography are collected and applied to Amicon Ultra centrifugal filter device (10,000 MWCO) for buffer exchange and concentrating
- Rinse the filter device with Milli-Q water prior to sample application, centrifuge for 10 min at 4 °C and 4000 x g in a swinging-bucket rotor

NOTE: do not let the membrane dry out! Remove concentrated sample immediately after centrifugation for best recovery!

- Apply the sample and centrifuge until the liquid has passed the membrane (~ 15 min), leave at least 500 µl protein concentrate in the filter unit to avoid protein precipitation
- Discard the flow through
- add buffer (20 mM Tris, pH8.0, 150 mM NaCl, 0.5 mM EDTA, 5 mM MgCl2, and 1 mM ATP) on top of the concentrate

Citation: Christin KÃÂ¶bler, Nicolas M Schmelling, Alice Pawlowski, Philipp SpÃÂ¤t, Nina Scheurer, Lutz LCB Berwanger, Ilka Axmann, Annegret Wilde (07/05/2021). Purification of recombinant Synechocystis KaiA3-His6 with PureProteome Nickel Magnetic Beads. https://dx.doi.org/10.17504/protocols.io.bu5bny2n

to the max. fill volume of the filter device and centrifuge again at 4°C and 4000 x g until max. 500 µl concentrate remains on the filter

- repeat the washing until the buffer exchange is complete
- leave ~ 1 ml final protein concentrate and transfer the protein solution to a fresh Eppendorf tube
- check for protein precipitation and remove precipitate by centrifugation
- measure protein concentration with Bradford reagent and store sample (100 µl aliquots) at – 80°C (long term storage) or at -20°C if you plan to perform experiments directly after

SDS-PAGE

7

- analyze the samples you have taken during the expression and purification procedure by SDS-PAGE (10% to 12% acrylamid)
- boil the samples at 95°C in a heat block for 5 min, vortex for 20 sec, and incubate once more at 95°C for ~ 2-5 min
- spin down the samples for 1' at full speed in a table centrifuge
- apply 10 µl sample/lane
- marker: 5 µl prestained PAGE-ruler, Thermo Scientific (use directly without heating)

Citation: Christin KÃÂ¶bler, Nicolas M Schmelling, Alice Pawlowski, Philipp SpÃÂ¤t, Nina Scheurer, Lutz LCB Berwanger, Ilka Axmann, Annegret Wilde (07/05/2021). Purification of recombinant Synechocystis KaiA3-His6 with PureProteome Nickel Magnetic Beads. https://dx.doi.org/10.17504/protocols.io.bu5bny2n