JUL 11, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.meac3ae

External link:
<https://doi.org/10.1128/JB.00478-19>

Protocol Citation: Anika Wiegard, Christin Köbler, Katsuaki Oyama, Anja K. Dörrich, Chihiro Azai, Kazuki Terauchi, Annegret Wilde, Ilka Maria IM Axmann 2019. Heterologous expression and affinity purification of Strep-tagged (KaiC) proteins. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.meac3ae>

MANUSCRIPT CITATION: Wiegard A, Köbler C, Oyama K, Dörrich AK, Azai C, Terauchi K, Wilde A, Axmann IM, Array. *Journal of Bacteriology* 202(4). doi: [10.1128/JB.00478-19](https://doi.org/10.1128/JB.00478-19)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Heterologous expression and affinity purification of Strep-tagged (KaiC) proteins

Journal of Bacteriology

Anika Wiegard¹, Christin Köbler², Katsuaki Oyama³, Anja K. Dörrich⁴, Chihiro Azai^{5,3}, Kazuki Terauchi^{5,3}, Annegret Wilde², Ilka Maria IM Axmann⁶

¹Heinrich-Heine Universität Düsseldorf;

²Institute of Biology III, Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany;

³Graduate School of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan;

⁴Institute for Microbiology and Molecular Biology, Justus-Liebig University, 35392 Giessen, Germany;

⁵College of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan;

⁶Institute for Synthetic Microbiology, Cluster of Excellence on Plant Sciences (CEPLAS), Heinrich Heine University Duesseldorf, 40225 Duesseldorf, Germany

Axmann Lab

CyanoWorld

Anika Wiegard

Heinrich-Heine Universität Düsseldorf

ABSTRACT

This protocol can be used to:

- (i) express Strep-tagged proteins in *E. coli*
- (ii) lyse cells
- (iii) purify Strep-tagged proteins via gravity flow affinity chromatography using either Strep-tactin XT superflow or Strep-Tactin resin

Protocol status: Working
We use this protocol and it's working

Created: Dec 23, 2017

Last Modified: Jul 11, 2019

PROTOCOL integer ID:
9378

Keywords: pASK, Strep-tag, affinity chromatography, KaiC, recombinant protein, heterologous expression, cell lysis, protein purification

MATERIALS

MATERIALS

- ☒ NaOH **Contributed by users**
- ☒ Magnesium chloride hexahydrate **Sigma Aldrich**
- ☒ Tris(hydroxymethyl)aminomethane **Sigma Aldrich Catalog #252859-500G**
- ☒ NaCl **Contributed by users**
- ☒ Lysozyme **Sigma Aldrich Catalog #12671-19-1**
- ☒ Benzonase **Merck Millipore Catalog #101654**
- ☒ Roche Complete Protease Inhibitor EDTA-Free tablets **Sigma Aldrich Catalog #5056489001**
- ☒ Ampicillin sodium salt **Millipore Sigma Catalog #A0166**
- ☒ HCl **Contributed by users**
- ☒ 14 Dithiotreitol (DTT) **Carl Roth Catalog #6908.1**
- ☒ Adenosin-5-triphosphate disodium salt (ATP) **Carl Roth Catalog #HN35.1**
- ☒ Quick Start™ Bradford 1x Dye Reagent **Bio-rad Laboratories Catalog #5000205**
- ☒ LB broth (Lennox) **Carl Roth Catalog #X964.1**
- ☒ pASK-IBA5plus **Contributed by users Catalog #2-1404-000**
- ☒ Rosetta-gami™ B (DE3) Competent Cells - Novagen **Contributed by users Catalog #71136**
- ☒ Chloramphenicol **Carl Roth Catalog #3886.2**
- ☒ D()Biotin **Contributed by users Catalog #2-1016-002**
- ☒ Anhydrotetracycline **Contributed by users Catalog #2-0401-001**

STEP MATERIALS

- ☒ Strep-Tactin®XT Superflow® 50% suspension **Contributed by users Catalog #2-4010-010**
- ☒ Strep-Tactin® Sepharose® 50% suspension **Contributed by users Catalog #2-1201-010**
- ☒ D()Biotin **Contributed by users Catalog # 2-1016-002**
- ☒ 10x Buffer R Strep-Tactin® Regeneration Buffer with HABA **Contributed by users Catalog #2-1002-100**
- ☒ Desthiobiotin **Contributed by users Catalog #2-1000-001**

heterologous protein expression in E.coli

1

transformation

- transform *E.coli* expression cells (e.g. Rosetta gamiB (DE3,) or Rosetta gami2 (DE3)) with your pASK based expression plasmid

2 pre-culture:

- inoculate LB medium containing 75-100 µg ampicillin ml⁻¹ (optional: plus 30 µg chloramphenicol ml⁻¹) with resulting transformants (use e.g. 200 ml LB)
- incubate over night at 37 °C and 200-250 r.p.m.

3 expression culture:

- inoculate LB medium containing 75-100 µg ampicillin ml⁻¹ (optional: plus 30 µg chloramphenicol ml⁻¹) with 4-10 % volume of the pre-culture (add e.g. 200 ml pre-culture to 1.8 l LB)
- incubate at 37 °C and 200-250 r.p.m. until OD_{600nm} = ~0.5

Note: use erlenmeyer flasks with a volume of at least 4l to ensure sufficient aeration.

4 induction of protein expression:

- when cell density reaches OD_{600nm} = ~0.3-0.7 (optimal 0.5) add 200 µl of 2 mg anhydrotetracycline ml⁻¹ (final concentration 200 ng anhydrotetracycline ml⁻¹)
- choose optimal expression condition (has to be tested for each protein of interest), e.g.: over night at 18-25 °C and 200-250 r.p.m. or at 35-37 °C and 200-250 r.p.m. for 3.5.-5.5 hours

cell harvest

- ## 5
- spin cells for 10 min at 4°C and 4000g
 - discard supernatant
 - keep cells on ice

00:10:00 centrifugation

cell disruption

6 enzymatic lysis by lysozyme:

- weight cell pellets
- resuspend cells in ice-cold buffer W [20mM Tris/HCl (pH8), 150 mM NaCl, 2 mM DTT (*only for KaiC proteins: 5 mM MgCl₂, 1 mM ATP*)] including protease inhibitors (e.g. protease inhibitor cocktail, Roche) using a paint brush. (use 3 ml buffer per g cells)
- add a spatula tip's worth of lysozyme (or add lysozyme stock solution to a final concentration of 1 mg lysozyme ml⁻¹)
- add 125 U benzonase
- incubate on ice for 30 min

Note: you can also use 50 mM Tris in buffer

Note: you can skip enzymatic lysis step and perform longer sonication instead

00:30:00 incubation on ice

7 sonication:

- sonicate the cell suspension for 6 min on ice using e.g. a Bandelin sonopuls homogenizer and the following parameters
- Note: During sonication, the temperature of the cell suspension should be kept below 15 °C

tip	KE76
cycle	1 (0.1 sec active cycle, 0.9 sec passive cycle)
output	60 %

Note: if you did not perform enzymatic lysis as described before, use alternative prolonged sonication conditions (e.g. alternation of 10 sec pulse and 10 sec pause for 25 minutes with 30 % output)

00:06:00 sonication

8 clarification of the lysate:

- centrifuge the resulting lysate for 20 min at 4 °C and 23000 g to remove insolubles
- keep the resulting supernatant (= soluble proteins)

Note: Many conical centrifugation tubes cannot withstand centrifugation of 23000g. If you want to use them, you can reduce centrifugal force, while increasing centrifugation time.

00:20:00 centrifugation

affinity purification

9 preparation of affinity column (at 4°C or room temperature)

- pour 3 ml Strep-tactin XT superflow resin (or Strep-Tactin Sepharose) in an appropriate glass column
- equilibrate with 30 ml ice-cold buffer W
- alternatively: equilibrate with 25 ml ice-cold buffer W and subsequently with 5 ml ice-cold buffer W including protease inhibitor

Note: if resin was stored in buffer R before, the colour will change from orange to white

Strep-Tactin®XT Superflow® 50% suspension **Contributed by users Catalog #2-4010-010**

Strep-Tactin® Sepharose® 50% suspension **Contributed by users Catalog #2-1201-010**

10 protein binding (at 4°C or room temperature)

- apply soluble proteins to your column
- collect the flow through

11 washing (at 4°C or room temperature)

- wash column with 15-50 ml ice-cold buffer W including protease inhibitors (at 4°C or room temperature)

12 elution from Strep-Tactin XT superflow resin (if you use Strep-Tactin Sepharose proceed with step 13 instead) (at 4°C or room temperature)

- elute proteins with 9 ml ice cold buffer BXT (buffer W + 50 mM D(+)biotin)
- collect 6 fractions of 1.5 to 2ml

D(+)Biotin **Contributed by users Catalog # 2-1016-002**

13 elution from Strep-Tactin Sepharose (if you use Strep-tactin XT superflow resin skip this step and move on to step 14) (at 4°C or room temperature)

- elute proteins with 15-30 ml ice cold buffer W + 2.5 mM desthiobiotin
- collect fractions of 1 to 2ml

Desthiobiotin **Contributed by users Catalog #2-1000-001**

14 regeneration of Strep-Tactin XT superflow resin (if you use Strep-Tactin Sepharose proceed with step 15 instead) (at room temperature)

- wash with 15 ml 10 mM freshly prepared NaOH
- optional: regeneration can be tested by adding buffer R (100 mM Tris-Cl, 150 mM NaCl, 1 mM EDTA, 1 mM HABA (hydroxy-azophenyl-benzoic acid)). An orange-shift indicates successful regeneration

Note: you cannot use buffer R for regeneration of Strep-tactin XT superflow resin

10x Buffer R Strep-Tactin® Regeneration Buffer with HABA **Contributed by users Catalog #2-1002-100**

15 regeneration of Strep-Tactin Sepharose (if you use Strep-tactin XT superflow resin, you already finished regeneration with step 14) (at room temperature)

- wash with buffer R (0.1 M Tris-Cl, 150 mM NaCl, 1 mM EDTA, 1 mM HABA (hydroxy-azophenyl-benzoic acid)) until agarose turns orange

16 qualitative analysis of eluate fractions:

- for each fraction, pipette 80 µl of Bradford solution in a well of a 96 well plate
- add 5-20 µl of your fraction
- colour change to blue indicates successful elution of proteins --> keep those fractions

- control quality of eluted protein by separation via SDS-PAGE (optional: analyse aliquots of lysate, flow through and washing steps in parallel)

17 buffer exchange:

- a) perform size exclusion chromatography or
- b) exchange buffer using centrifugal concentrators
 - mix all eluate fractions of sufficient protein quality and transfer them to a disposable centrifugal concentrator
 - concentrate protein by centrifugation
 - add your desired buffer
 - concentrate again
 - repeat this step until the buffer is completely exchanged

Note: choose the molecular cut-off of the concentrator and centrifugal force according to the manufacturer's instructions.