Jul 11, 2019

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

Journal of Bacteriology

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

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DOI: https://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 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

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

Created: December 23, 2017

Last Modified: July 11, 2019

Protocol Integer ID: 9378

Keywords: pASK, Strep-tag, affinity chromatography, KaiC, recombinant protein, heterologous expression, cell lysis, protein purification, affinity purification of strep, tagged protein, proteins via gravity flow affinity chromatography, kaic protein, affinity purification, proteins this protocol, strep, gravity flow affinity chromatography, protein, purification, kaic

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

Materials

MATERIALS

- NaOH
- Magnesium chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich)**
- Tris(hydroxymethyl)aminomethane **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**252859-500G
- NaCl
- Lysozyme **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**12671-19-1
- Benzonase **Merck Millipore (EMD Millipore)** **Catalog #**101654
- Roche Complete Protease Inhibitor EDTA-Free tablets **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**5056489001
- Ampicillin sodium salt **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A0166
- HCl
- 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 **Catalog #**2-1404-000
- Rosetta-gami™ B (DE3) Competent Cells - Novagen **Catalog #**71136
- Chloramphenicol **Carl Roth** **Catalog #**3886.2
- D( )Biotin **Catalog #**2-1016-002
- Anhydrotetracycline **Catalog #**2-0401-001

STEP MATERIALS

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

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- 10x Buffer R Strep-Tactin® Regeneration Buffer with HABA **Catalog #**2-1002-100
- Lysozyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**12671-19-1
- Roche Complete Protease Inhibitor EDTA-Free tablets **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**5056489001

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 $OD600_{nm}$ = ~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 $MgCl_2$, 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 clarifaction 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 **Catalog #**2-4010-010

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

10 protein binding (at 4°C or room temperature)
- apply soluble proteins to your column
- collect the flow though

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 **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 **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 **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.