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

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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

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

NAME	CATALOG #	VENDOR
Anhydrotetracycline	2-0401-001	

STEPS MATERIALS

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

heterologous protein expression in E.coli

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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.