



Ribosome Purification for OnePot PURE cell-free system 👄

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1 Works for me

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ABSTRACT

In this protocol we explain the procedure of ribosomes purification with the method of hydrophobic interaction chromatography (HIC) so they can be used for a PURE cell-free system.

EXTERNAL LINK

https://pubs.acs.org/doi/10.1021/acssynbio.8b00427

MATERIALS TEXT

Material/Consumables:

- Liquid LB medium (pH 7) Autoclaved
- Ethanol 70%
- A19 E. Coli strain
- Glycerol stock 40%
- Suspension buffer
- β-Mercaptoethanol
- Milli-Q water
- 0.22μm PES membrane filter
- 20% Ethanol
- Buffer C
- Buffer B
- NaOH (1M)
- Acetic Acid (0.1M)
- Cushion buffer
- Ribosomes buffer

Equipment:

- Flame
- 11 Erlenmeyer Flask narrow mouth with baffles Autoclaved
- 2 * 5l Erlenmeyer Flask narrow mouth with baffles Autoclaved
- OD600 Spectrophotometer
- Centrifuge
- Sonicator
- 3*250ml Beaker
- Syringe
- 2*5ml HiTrap Butyl HP Column
- Ultracentrifuge
- Magnetic stirrer

SAFETY WARNINGS

When handling β -Mercaptoethanol the researcher should work in a chemical hood and wear protective glasses.

BEFORE STARTING

The recipes of the buffers used in this protocol can be found here:



Small cell culture

1

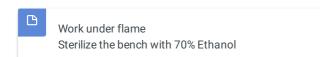


Add 100ml of LB medium to the 1L Erlenmeyer flask

- 2 Inoculate the LB with 10µl of A19 E. coli strain kept in glycerol stock
- 3 Incubate overnight (at least 12 hours) at 37°C rotating at 250rmp

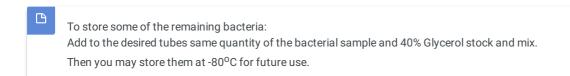
Large cell culture

4



Add 2I of LB medium to each of the two 5I flasks

5 Inoculate the flasks with 30ml of the A19 E. Coli culture each



- 6 Incubate the flasks for 2 hours at 37°C rotating at 180rpm
- 7 Test the bacterial sample with OD600 Spectrophotometer

7.1	Add 1ml of the sample to a cuvette and measure it
7.2	If the measurement is lower than 1.0 continue incubating and after the appropriate time repeat the measurement
	Take into account that E.coli has a doubling time of about 20-30 minutes
Cell sı	uspension and lysis
8	
	Precool the centrifuge to 4°C before using as the sample is quit sensitive
	Centrifuge the bacterial sample for 20min with 6000 RCF at 4°C
9	Remove the supernatant from the tubes .The cells should be shaped into a pellet on the wall of the tube
10	Add $25\mu l$ of $\beta\text{-Mercaptoethanol}$ to $50ml$ of Suspension buffer
	The final concentration of β -Mercaptoethanol in the buffers should always be 7mM before each use
11	Use 40ml of the buffer to resuspend the cells and then with the another 10ml wash the tubes to collect any cells left attached to the tubes
12	Gather the sample into two tubes (appr. 25-35ml liquid in each)
	At this point we can store the samples at -80°C overnight
13	
	Clean the sonicator by applying one operation cycle to milli-Q water
	Sonicate each tube on ice with 20s on 20s off for an active time of 4min at 70% of total power
	It is very important to keep the cells on ice to avoid damaging the proteins as they are very sensitive to temperature



Centrifuge the samples for 20min with 20000RCF at 4°C



- 15 Collect the sample into a single beaker and measure the total volume (e.g. 60ml)
- Prepare the same quantity of High salt suspension buffer by adding 2000x concentration of β -Mercaptoethanol (e.g. add 30 μ l of β -Mercaptoethanol to 60ml of High salt suspension buffer) and mix the solutions.

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Centrifuge the samples for 20min with 20000RCF at 4°C



 $\label{eq:peaker} \textbf{18} \quad \text{Filter the supernatant through a syringe filter with a 0.22} \mu \text{m PES membrane and gather the sample to a single Beaker}$

Ribosome purification

19 Preparation of the purifier: Clean the input filter of the AKTA Purifier

Set the ambient temperature to 4°C

Connect three HiTrap Butyl HP column (5ml) in series to the purifier

Preparation of the buffers: Add 75 μ l of β -Mercaptoethanol to 150ml of Buffer C Add 60 μ l of β -Mercaptoethanol to 120ml of Buffer D

20 Remove the ethanol in the columns with 45ml of Milli-Q water



21 Equilibrate columns with 60 ml buffer C

22	Add approximately 90% of the lysate sample to the system	
23	Wash the column with 45mL of wash buffer 1 (100% buffer C)	
24	Wash the column with 75mL of wash buffer 2 (80 buffer C, 20% buffer D)	
25	Elute the ribosomes with 60ml of elution buffer 1 (50% buffer C, 50% buffer D) and then with 60ml of elution buffer 2 (100% buffer D)	
26	The eluted ribosomes are collected into tubes of 4.5ml	
27	Pick the tubes that have the biggest absorbance in wave length 280nm	
Colur	nn recovery	
28	Wash with 45ml of milli-Q water	
29	Wash with 45ml of NaOH (1M)	
30	Wash with 45ml of milli-Q water	
31	Wash with 45ml of Acetic Acid (0.1 M)	
32	Wash with 45ml of milli-Q water	
33	Equilibrate with 45ml of Ethanol 20%	
	After the cleaning the column is stored at 4°C in 20% ethanol and can be reused	
Ribosome collection		
34		
	We will use two tubes for Ultracentrifuge	
	Add 14μl of β-Mercaethanol to 28ml of Cushion buffer and mix	

35	Place 14ml of Cushion Buffer to each of the Ultracentrifuge tubes	
36	Carefully place the ribosomes solution on top of the cushion buffer and make sure that we still see the separation layer	
37	Ultracentrifuge for 16 hours with 24000RPM (100000RCF) at 4°C	
38	Discard the liquid	
	The pellet of ribosomes might not be visible at this point	
39	Add 2μl of β-Mercaethanol to 4ml of Ribosome Buffer and mix	
40	Add $500\mu l$ of Ribosome buffer to the wall of the tube and slightly spin to wash the cushion buffer on each tube and then discard the liquid	
41	Repeat step No 39	
42	Add 100µl of Ribosome buffer to each tube and resuspend the cells with a magnetic stirrer on ice for 10min at 200rpm	
43	Collect the samples to a single tube (Tube No1)	
44	Wash the centrifuge tubes with $100\mu l$ of Ribosome Buffer and add that to another tube (Tube No2)	
Measurements and concentration		
45	Load the solution to a 0.5mL Amicon Ultra filter with 3kDa molecular weight cutoff and centrifuge at 14000RCF for 10min at 4	
	Use two separate filter for Tube No1 and No2	
46	Dilute $1\mu l$ of the concentrated solution to $99\mu l$ of ribosome buffer (or milli-Q water?) and measure the absorbance at 260nm	
47	Optionally , if the absorbance of Tube No2 is more than 10% of the one measured for the Tube No1 you can mix the two samples and repeat steps 44 and 45	



Absorbance of 10 corresponds to 23uM concentration

Adjust the final concentration 10uM by dilution with ribosome buffer

Storage

Aliquote the final solution according to your needs (0.9μ l are needed for one 5μ l reaction) and store at -80°C

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