Extraction of total RNA from *E. coli* cells

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1 Works for me

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ABSTRACT

The protocol is used for the extraction of total RNA from *E. coli* cells. It is based on the method described by Chomczynski and Sacchi, 1987 (https://doi.org/10.1016/0003-2697(87)90021-2). Total RNA is isolated from cells expressing small regulatory RNAs (trRNAs) either constitutively (E. coli W3110) or after induction with anhydrotetracycline (E. coli W1310 Z1).

MATERIALS

NAME	CATALOG #	VENDOR
Roti-Aqua-P/C/I	X985.2	Carl Roth
Roti-C/I	X984.2	Carl Roth
NucleoZOL	REF 740404.200	Macherey and Nagel
Roti-Aqua-Phenol for RNA extraction	A980.1	Carl Roth
LB-Medium (Lennox) vegetal	0155.1	Carl Roth
IPTG	CN08.1	Carl Roth
Anhydrotetracycline	2-0401-001	

MATERIALS TEXT

additional solutions/buffers:

- Antibiotic stock solutions:
  Kanamycin (Km): 25 mg/ml
  Spectinomycin (Spec): 100 mg/ml
- Inducer stock solutions: 20 µg/ml Anhydrotetracycline (aTc) in EthOH, 10 mM Isopropyl-ß-D-thiogalactopyranosid (IPTG) in EthOH and a mixture of 20 µg/ml aTc and 10 mM IPTG in EthOH
- Ethanol
- Isopropanol
- 3 M Na-Acetat, pH 5.2
- RNAse free molecular grade water (DEPC-treated)

SAFETY WARNINGS

Phenol is toxic! Work under the hood, wear protective gloves (Nitril) and change gloves immediately after contamination. Collect solid and liquid waste in special waste containers.

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BEFORE STARTING

RNA is sensitive to degradation! Wear gloves, keep samples on ice when possible, use filter-tips and RNase free reagents. Pre-cool centrifuges and store isolated RNA-samples immediately at -20 or -80°C.

incubation of cells

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- freshly transform *E. coli* cells with plasmids encoding trRNA sequences or empty vector
- inoculate 3 ml LB-vegetal medium + antibiotic (25 µg/ml Km for W1310, 25 µg/ml + 100 µg/ml Spec for W1310Z1) in culture tubes with a single colony
- incubate o/n at 37°C and 230 rpm

for constitutive RNA-synthesis (*E. coli* W3110):

- dilute 1:100 in fresh medium with 25 µg/ml Km and incubate until OD600 reaches 0.7 - 1.0 (in culture tubes or 6 well plates), proceed to step 2

for induced RNA-synthesis (*E. coli* W3110Z1):

- dilute 1:50 in fresh medium (200 µl o/n culture in 10 ml medium + antibiotic in 100 ml Erlenmeyer flask) and incubate until OD600 reaches 0.4 - 0.6
- if you have several different cultures that do not grow at same speed: store samples on ice until the last one reaches the defined OD (keep that one on ice as well for 10 min)
- meanwhile prepare 12 well plates with inducer: pipet in each well of a row 10 µl of either
  1) EthOH,
  2) aTC (= 200 ng/ml final concentration)
  3) IPTG (= 100 µM final concentration)
  4) aTc + IPTG (200 ng/ml, 100 µM final concentration)
- add 1 ml of culture and incubate at 37°C and 230 rpm for 1 h
- proceed with step 2

stop of RNA synthesis

2

- work under the hood
- mix 1 ml of cells with 200 µl 'stopmix'- solution (5 % phenol in ethanol) in a 2 ml safe lock tube tube → stops RNA production in the cells
- centrifuge for 5 min at 4°C and 14000 x g
- discard the supernatant and resuspend the pellet in 1 ml NucleoZOL (Macherey and Nagel), place on ice (better for rapid freezing: dry ice or liquid nitrogen)

→ store cells at -20 or -80 °C (only for a short time, maximum 2 weeks) or proceed to next step

RNA-isoloation

3

- incubate the sample at 65 °C and 250 rpm (Thermomixer) for 10 min
- mix with 400 µl Phenol-Chloroform/Isoamylalcohol (Roti®-)Aqua-P/C/I) by inverting for 10 s
- centrifuge at 4°C for 10 min at 14000 x g
- transfer aqueous (upper) phase to a fresh 1,5 ml safe lock reaction tube, work on ice
- mix with 450 µl Chloroform/Isoamylalcohol (Roti®-C/I)
- centrifuge at 4°C for 10 min at 14000 x g
- transfer aqueous (upper) phase to a new reaction tube and add 1 Vol. icecold Isopropanol + 1/10 vol (e.g. 20 µl for 200 µl Isopropanol) 3 M Na-Acetat (pH 5.2), mix and store at least 30 min at -20 °C or -80 °C
- centrifuge at 4°C for 30 min at 14000 x g
- remove the supernatant (take care of the RNA-pellet) and add 350 µl of icecold 75% Ethanol
- centrifuge for 5 min at 4°C and 15000 rpm
- add again 350 µl of icecold 75% etahnol and centrifuge for 5 min at 4°C and 15000 rpm
- remove the supernatant and dry the pellet at room temperature for ca.15 min
- resuspend the pellet in 30 µl Molecular Biology Grade Water and store at -80°C

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