RNA extraction from Escherichia coli Version 3

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Abstract

RNA extraction from E. coli cells based on the method described by Chomczynski and Sacchi, 1987

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Guidelines

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.

Before start

Always keep your samples on ice!

Materials

Roti®-C/I by Carl Roth
Roti®-P/C/I by Carl Roth

Protocol

Cell prepartion

Step 1.

- mix 1 ml of cells with 200 µl 'stopmix'- solution (5 % phenol in ethanol) in a 2 ml tube → stops RNA production in the cells

SAFETY INFORMATION

Wear safety gear

Cell prepartion

Step 2.

- 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 dry ice
- proceed to next step or store cells at -20 or -80 °C

TEMPERATURE
4 °C Additional info:

RNA-isolation

Step 3.

- incubate the sample at 65 °C and 250 rpm (Thermomixer) for 10 min
- mix with 400 µl Chloroform/Isoamylalcohol (Roti®-C/I) by inverting for 10 s

TEMPERATURE
65 °C Additional info:

RNA-isolation

Step 4.

- centrifuge at 4°C for 10 min at 14000 x g
- transfer aqueous phase to a new reaction tube, work on ice
- mix with 450 µl Penol/Chloroform/Isoamylalcohol (Roti®-Aqua-P/C/I)
- centrifuge at 4°C for 10 min at 14000 x g
- transfer aqueous phase to a new reaction tube and add 1 Vol. icecold Isopropanol + 20 µl 3 M Na-Acetat (pH 5.2) and mix

TEMPERATURE
4 °C Additional info:
SAFETY INFORMATION
Wear safety gear!

RNA-isolation

Step 5.

- leave RNA at least 30 min at -20 °C or store over night

RNA-isolation

Step 6.

- 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% etahnol

TEMPERATURE
4 °C Additional info:

RNA-isolation

Step 7.

- centrifuge at 4°C for 5 min at 14000 x g

TEMPERATURE

4 °C Additional info:

RNA-isolation

Step 8.

- add 350 µl of icecold 75% ethanol
- centrifuge for 5 min at 4°C and 14000g

TEMPERATURE

4 °C Additional info:

RNA-isolation

Step 9.

- remove the supernatant and dry the pellet at room temperature for ca.15 min
- resuspend the pellet in 30 µl molecular biology grade water or TE-buffer (10 mM Tris/HCL pH 8.0 , 1 mM EDTA)

Warnings

Phenol is toxic! Work under the hood, always wear protective gear and change contaminated gear immediately. Collect solid and liquid waste in special waste containers.