RNA extraction from Escherichia col V.1

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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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Chomczynski and Sacchi, 1987

KEYWORDS

RNA extraction, RNA, Ecoli

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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.

MATERIALS TEXT

MATERIALS

Roti®-C/I **Carl Roth**

Roti®-P/C/I **Carl Roth**

SAFETY 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.

BEFORE STARTING

Always keep your samples on ice!

Cell prepartion

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

> Wear safety gear

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

00:05:00

4 °C

RNA-isolation

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

00:10:00

65 °C

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

00:10:00 **2nd centrifugation step**

00:10:00 **1st centrifugation step**

4 °C

> Wear safety gear!

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

00:30:00

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

00:30:00

4 °C

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

00:05:00

4 °C

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8
- add 350 µl of icecold 75% etahnol
- centrifuge for 5 min at 4°C and 14000g

00:05:00

4 °C

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)

00:15:00

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