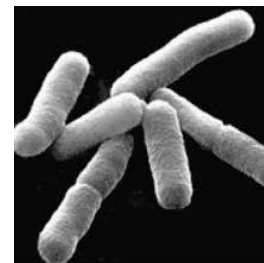


Nov 24, 2017 Version 2

RNA extraction from Escherichia coli V.2

DOI

dx.doi.org/10.17504/protocols.io.kykcxuw



Alice Pawlowski¹, Lutz LCB Berwanger¹

¹Heinrich-Heine Universität Düsseldorf

Axmann Lab



Lutz LCB Berwanger

Heinrich-Heine Universität Düsseldorf

OPEN  ACCESS



DOI: dx.doi.org/10.17504/protocols.io.kykcxuw

Protocol Citation: Alice Pawlowski, Lutz LCB Berwanger 2017. RNA extraction from Escherichia coli. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.kykcxuw>

Manuscript citation:

Chomczynski and Sacchi, 1987

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: November 24, 2017

Last Modified: March 27, 2018

Protocol Integer ID: 8940

Keywords: RNA extraction, RNA, Ecoli



Abstract

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

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

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 start

Always keep your samples on ice!



Cell preparation

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

Safety information

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 μ l 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 μ l 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 μ l 3 M Na-Acetate (pH 5.2) and mix

⌚ 00:10:00 2nd centrifugation step

⌚ 00:10:00 1st centrifugation step

🌡 4 °C

Safety information

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% ethanol

00:30:00

4 °C

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

00:05:00

4 °C

- 8
- add 350 µl of icecold 75% ethanol
 - 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