RNA extraction from E. coli

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Oct 07, 2022

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dx.doi.org/10.17504/protocols.io.j8nlkw44wl5r/v1

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DISCLAIMER

ABSTRACT

RNA extraction is a fundamental step in multiple experiments, for example, qPCR. This protocol helps conduct a simple RNA extraction procedure.

DOI

dx.doi.org/10.17504/protocols.io.j8nlkw44wl5r/v1

PROTOCOL CITATION

An.Huang 2022. RNA extraction from E. coli. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkw44wl5r/v1

LICENSE

CREATED

Oct 06, 2022

LAST MODIFIED

Oct 07, 2022

PROTOCOL INTEGER ID

70925

MATERIALS TEXT

Buffer LY (added 1% volume of dithiothreitol), Buffer RB, RNA Wash Buffer, DEPC-Treated ddH_2O, RNA Columns, DNA Clearance Column, Collection Tubes, 1.5 mL RNase-free microfuge tube, Lysozyme buffer (0.4 mg/mL)

DISCLAIMER:

Preparation for experiment

1 Grow an overnight bacterial culture in the appropriate media at an appropriate temperature.

2 In the following day, take **1 mL** from overnight culture and add into **10 mL** LB media. Grow until the OD600 reads at 0.6-1.0.

RNA extraction 17m 30s

3 Harvest 1.5 mL culture (< 5x10%) by centrifugation at **3.000 rpm, 00:10:00** for 10 min 10m in a 1.5 mL microcentrifuge tube.

Discard all supernatant.

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You may use a pipette to remove the remaining liquid at the bottom of the tube.

5 Resuspend the pellet in **100 µL** freshly prepared Elution Buffer (10mM Tris-HCL pH 8.5) containing lysozyme (0.4 mg/mL lysozyme for Gram negative bacteria). Mix by tapping gently.

6 Incubate the resuspended pellet at room temperature for 3-5 min for Gram-negative bacteria.

7 Add 400 µL Buffer LY. Mix gently.

8 Transfer the cleared lysate to a DNA Clearance column pre-inserted in a 2 mL Collection Tube. 2m
Centrifuge at **13.000 rpm, 00:02:00** . Discard the DNA Clearance column and save the flow-through.

9 Transfer flow-through to the RNA binding column. Add 0.5 volume 100% ethanol to the lysate.

For example: 250 µL 100% ethanol for 500 µL.

10 Centrifuge at **13000 rpm, 00:01:00** . Discard the collection tube with the flow through and 1m
put the column back to a new collection tube.

11 Add **500 µL** Buffer RB to the column and centrifuge at **13.000 rpm, 00:00:30** . Discard 30s
the flow-through.

12 Add another **500 µL** RNA Wash Buffer to the column and centrifuge at 30s
13000 rpm, 00:00:30 . Discard the flow-through.

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Ethanol should be first added into RNA Wash Buffer before use.

13 Add another **500 µL** RNA Wash Buffer to the column and centrifuge at **13000 rpm, 00:00:30** . Discard the flow-through and collection tube, put the column into a new collection tube. 30s

14 Centrifuge the column at **13000 rpm** , with the lid open, for another **00:01:00** . 1m

15 Place the column to a RNase-free 1.5 mL tube, add 50-100 µL DEPC treated ddH_2O to the column and centrifuge at **13000 rpm, 00:02:00** . 2m

The RNA is in the flow-through.

16 Store the RNA solution at **-20 °C** .

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