



Oct 07, 2022

RNA extraction from E. coli

An.Huang¹¹XJTLU

1 Works for me

 Sharedx.doi.org/10.17504/protocols.io.j8nlkw44wl5r/v1 An.Huang

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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

————— This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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 ddH₂O, RNA Columns, DNA Clearance Column, Collection Tubes, 1.5 mL RNase-free microfuge tube, Lysozyme buffer (0.4 mg/mL)

DISCLAIMER:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK


The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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 in a 1.5 mL microcentrifuge tube.

Discard all supernatant.


4

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 put the column back to a new collection tube.^{1m}


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

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

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 **30s**
 **13000 rpm, 00:00:30** . Discard the flow-through and collection tube, put the column into a new collection tube.

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 ddH₂O to the **2m**
column and centrifuge at  **13000 rpm, 00:02:00** .

The RNA is in the flow-through.

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