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RNA extraction from E. coli

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We use this protocol and it's working

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Abstract

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



Materials

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)

Troubleshooting




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 ($< 5 \times 10\%$) by centrifugation at  3.000 rpm, 00:10:00 for 10 min in a 1.5 mL microcentrifuge tube. 10m
- 4 Discard all supernatant.


Note



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



- 5 Resuspend the pellet in  100 μ L 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 μ L Buffer LY. Mix gently.
- 8 Transfer the cleared lysate to a DNA Clearance column pre-inserted in a 2 mL Collection Tube. Centrifuge at  13.000 rpm, 00:02:00. Discard the DNA Clearance column and save the flow-through. 2m
- 9 Transfer flow-through to the RNA binding column. Add 0.5 volume 100% ethanol to the lysate.

**Note**

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  13000 rpm, 00:00:30 . Discard the flow-through. 30s

Note

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

Note

The RNA is in the flow-through.



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