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RNA extraction from E. coli

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Protocol status: Working
We use this protocol and it's working

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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.

Materials

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)

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 (< 5×10%) 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 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. 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 ddH_2O 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 .