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RNA isolation

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We use this protocol and it's working

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Disclaimer

Abstract

RNA isolation for sample stored in TRIzol reagent

RNA isolation

23m

1 Sample in TRIzol

Equipment	
accuSpin Micro 17R refrigerated centrifuge	NAME
refrigerated microcentrifuge	TYPE
Fisher Scientific	BRAND
Micro17R	SKU

Prepare the centrifuge at 4°C and meanwhile thaw the frozen sample (in TRIzol, stored at -80°C) at room temperature for about 10-15 minutes.

2 Add chloroform at 20% of the sample volume to the sample tube (typically, 1 ml of TRIzol sample, so add 200 µl of chloroform in sample tube) and incubate at room temperature for 5 minutes. Mixing from time to time during the incubation

3 Centrifuge at 13,000 rpm, 4°C for 15 minutes.

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3.1 In a meanwhile, prepare the mixture of RNA precipitation as follow:
a. Isopropanol: 0.8 times the total volume of supernatant plus 400 µl DEPC water (typically, take 400 µl of supernatant after centrifugation (clear part), add 640 µl of isopropanol) per sample.
b. DEPC water: 400 µl per sample.
c. Glycoblue: 1-2 µl per sample.
- *Note: Always prepare 2-3 extra mixtures.*

4 Transfer 400 µl of supernatant after centrifugation (clear part) to a new Eppendorf tube - Carefully pipetting

5 Add 1,041 µl of the RNA precipitation mixture (400 µl DEPC water + 640 µl isopropanol + 1 µl Glycoblue) to the supernatant. Incubate on ice for 10-30 minutes.

6 Centrifuge at 13,000 rpm, 4°C for 15 minutes.

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7 Check if a blue pellet has formed at the bottom of the Eppendorf tube, then discard the supernatant and add 1 ml of 75% ethanol (*ETOH volume is proportional to TRIzol volume*).

8 Centrifuge at 13,000 rpm, 4°C for 5 minutes.

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9 Discard the supernatant, then add 1 ml of 75% ethanol and transfer everything to a new Eppendorf tube – *Ensure the pellet is transferred as well.*

10 Centrifuge at 13,000 rpm, 4°C for 5 minutes.

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11 Discard all ethanol and air-dry the pellet in the chamber for 5 minutes.

12 Add 20 µl of nuclease-free water to each Eppendorf tube

13 Prepare the RNA treatment mixture (10 µl per sample) of:
a. 6 µl of nuclease-free water.
b. 3 µl of DNase buffer.
c. 1 µl of DNase enzyme.
Note: Always prepare 1-2 extra mixtures.

14 Add 10 µl of the RNA treatment mixture to the sample and incubate at 37°C for 20 minutes on thermo shaker incubator

20m

15 Add 3 µl of DNase inactivation reagent and incubate for 2 minutes at room temperature.

16 Centrifuge at 13,000 rpm, 4°C for 3 minutes.

3m

Equipment	
accuSpin Micro 17R refrigerated centrifuge	NAME
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Fisher Scientific	BRAND
Micro17R	SKU

17 Transfer 25 µl of the supernatant to a 1.5 ml Eppendorf tube – *keep on ice until RNA measurement.*