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RNA isolation

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We use this protocol and it's working

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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.



Equipment

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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. 

Equipment

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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.  

Equipment

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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.



Equipment

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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

refrigerated microcentrifuge

TYPE

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.*