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RNA Extraction with Trizol V.1

Forked from RNA Extraction with Trizol

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

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Abstract

This is a protocol for RNA extractions (using Trizol). A maximum of 3 samples can be extracted at once using this protocol. The entire protocol should be run in the fumehood. This protocol is time- and temperature-sensitive, so read all instructions before attempting and make sure you are adequately prepared.

Guidelines

A maximum of 3 samples can be extracted at once using this protocol.

Materials

- RNase Away
- RNA free tubes (1.6 and 2 ul)
- Trizol
- 100% ethanol (new every time)
- RNase free water (new every time)
- Direct-zol RNA miniprep kit (Zymo research) ← contains all other reagents needed

Protocol materials

UltraPure ™ Distilled Water

Chloroform

Safety warnings

Trizol is BAD--conduct entire extraction protocol in the fume hood! You will need to move the centrifuge, bead basher, and other equipment into the hood before starting. Make sure you have icepacks available as this protocol is time- and temperature-sensitive.

Before start

Celean all surfaces, tools, and gloves with RNase Away. Don't forget to clean pipettes, plastic pipettes, ice blocks, centrifuge, etc.
Make sure buffers have been prepped if it's a new kit (instructions in kit for which reagents/how much to add to each).

RNA purification & HAV

1h 5m 30s

1

Add 200 µL of virus susoension to be extracted to a 1.5 mL tube

2 Lysis

5m

Add 800 µL of UltraPure ™ Distilled Water to tube (1 per sample). Mix thoroughly by brief pulse vortexing and incubate for 00:05:00 at room temperature.

3 Phase Separation

5m 30s

Add 160 µL of Chloroform to each tube. Shake vigorously for 00:00:30, then incubate for 00:02:00 to 00:03:00 at Room temperature.

4 Centrifugation

15m

Centrifuge samples 12000 rpm, 4°C, 00:15:00

5 Aqueous Phase Collection

After centrifugation, the mixture separates into lower red, phenol chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. Carefully remove 480 µL of the clear upper aqueous phase and transfer it to a new, labeled microcentrifuge tub 0 °C e. **Please note: Extract 80% of the aqueous RNA layer leaving 20% behind in the tube to prevent contaminating the aqueous layer.**

6 RNA Precipitation

20m

Add 400 µL of UltraPure ™ Distilled Water and 1 µL of UltraPure ™ Distilled Water co-precipitant to the aqueous phase. Mix by tilting **10** times and incub 800 µL ate for 00:20:00 at −20 °C.

7 RNA Pelleting

10m

Centrifuge for 12000 rpm, 4°C, 00:10:00

8 Ethanol Wash

A dark blue RNA pellet should be visible at the bottom of the tube if GlycoBlue was used.

Carefully discard the supernatant and wash the pellet with 800 µL of [M] 75 % (v/v) Ethanol

9 Wash Centrifugation 5m
Centrifuge for 12000 rpm, 4°C, 00:05:00

10 Ethanol Removal 5m
Discard the supernatant. Briefly centrifuge again to collect residual ethanol, then use a P200 pipette tip to remove the remaining ethanol without disturbing the pellet. Vacuum or air dry the RNA pellet for 00:05:00 . It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility.

11 1. RNA Resuspension
Resuspend the RNA pellet in 50 µL of UltraPure ™ Distilled Water by vortexing.
The dilution step was intentionally omitted to maximize sensitivity.

12 10) Store everything at -80 °C