Research Center for
Pharmaceutical Nanotechnology
Tabriz University of Medical Sciences
RCPN

RNA extraction procedure

PSR

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Workflow

RNA extraction → cDNA synthesis (RT-PCR) → Real-Time PCR → Data analysis

Detailed procedure

1. RNA extraction procedure

All Analysis requires firstly the extraction of RNA from a biological source of interest. Prepare all materials below then start!

Materials

product	Cat. No.
Trizol Reagent	66322
Chloroform	CL02002500-13226508
Isopropanol	-
1.5 mL micro-centrifuge tubes	-
Aerosol Resistant Tips (1000E, 200E, 20E, 10E)	-
Eppendorf Micro centrifuge	-
Ultrapure Dnase/Rnase-Free Distilled water	DW8520

Biohazard Consideration:

- Trizol is very toxic reagent. Working with Trizol should be in a fume hood and using personal protection equipment such as gloves, eye protection and etc.

Method

1. Remove the media (for adherent cells just remove whole media covering the cells but for the suspend cells first centrifuge the media in 130g for 5 minutes then remove the supernatant).
2. Add 400 µL Trizol reagent (for each plate containing 5×10^5 cells) and shake it for 20 min in room temperature to disrupting cells. (This amount of Trizol is used for each well of six-well plate).
3. Place the supernatant into a 1.5 mL Eppendorf tube.
4. Add 200 µL chloroform and shake the tube by hand for about 15 sec.
5. Incubate for 5 min in room temperature.
6. Centrifuge at 10,000 rpm for 5 minutes at 4°C.
7. Following centrifugation, the mixture in each tube separates into:
   - ✓ Top layer: colorless upper aqueous phase
   - ✓ Middle layer or interphase
   - ✓ Bottom layer: low red phase
8. Transfer the aqueous phase to a fresh tube.
9. Add 500 µL isopropanol and leave at room temperature for 5 min.
10. Centrifuge at 12,000 rpm for 10 minutes at 2-8°C.
11. Remove the supernatant.

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12. A pellet will be visible at the base of each tube. Let the pellets air-dry.
13. Add 20 µL DEPC water to the RNA pellet.
14. Transfer tubes to 70°C heat block for 2-3 minutes.
15. Use nanodrop instrument for measuring RNA concentration and purity. (The 260/280 ratio should be greater than 1.8)

Good luck!
☺

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