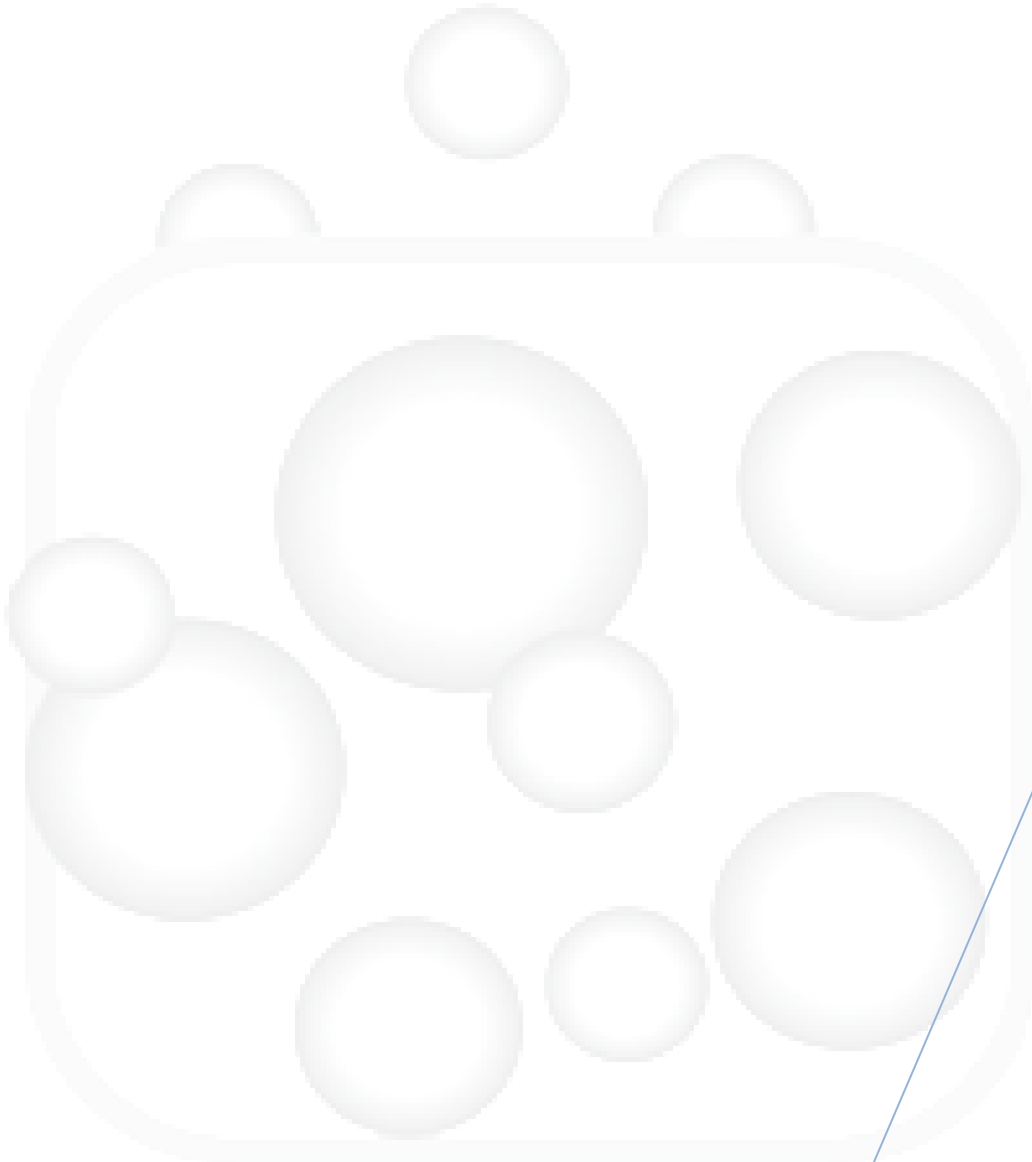




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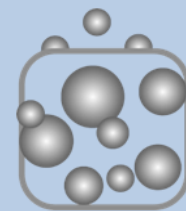
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RNA extraction procedure  
PSR

Golchin. A  
12/30/2015



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## Workflow

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

# Detailed procedure

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## 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  $\mu$ L Trizol reagent (for each plate containing  $5 \times 10^5$  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  $\mu$ L 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  $\mu$ L 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.

12. A pellet will be visible at the base of each tube. Let the pellets air-dry.
13. Add 20  $\mu$ L 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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Document name	RNA extraction procedure
Document arrangement	Mohanna Osali, Rahimeh Mousavi
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