

RNA Extraction from Cell Lines or Frozen Tissue

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National Institutes of Health

Reagents

Aggregates for Homogenizer

Brinkmann Instruments, Cat.9112007 and 9112012

Chloroform

Mallinckrodt, Cat.4440

Copolymer Centrifuge Tubes 15ml

Thomas Scientific, Cat.2610R22

Copolymer Centrifuge Tubes 50ml

Thomas Scientific, Cat.2610R54

Ethanol 100% and 75%

Homogenizer

Brinkmann Instruments, Cat.027-13-500-5

NaAcetate 3M pH 5.5

Quality Biological, Cat. 351-035-060

PBS, 1X 7.4

Invitrogen, Cat.10010-023

Trizol

Invitrogen, Cat.15596-018

Rnase Away

VWR, Cat.72830-022

RNeasy Maxi Kit

Qiagen, Cat.75162

Procedure

Trizol Extraction

The following protocol describes the procedure starting with a T175 flask, which should give around 200 µg total RNA at the end. However, volumes and procedures can be adjusted according to the Trizol protocol supplied with each Trizol reagent. One can alternatively start with homogenizing frozen tissue in Trizol reagent and continue with step 5.

1. Aspirate off cell culture media.
2. Wash surface twice with 20 ml 1X PBS and aspirate.

3. Add 10 ml Trizol and remove cells with a cell scraper.
4. Transfer cells into a 15 ml GREEN capped conical tube (one can freeze samples at this point at -80°C ; NOTE: change tubes when proceeding, otherwise cold tubes may crack while spinning).
5. Shake tubes for 15 sec.
6. Let sit at RT for > 5 min.
7. Add 2 ml chloroform with glass pipette (0.2 ml chloroform per 1 ml Trizol).
8. Shake for 15 seconds until it appears as a uniform pink suspension.
9. Allow to stand at RT for 10 min (phase separation should be visible; it goes faster if tubes are on ice).
10. Centrifuge at 8000 rpm for 15 min at 4°C .
11. Transfer ONLY the transparent, upper phase to a GREEN capped 50 ml conical tube (being careful NOT to transfer material from the interface layer- it's better to lose a little RNA than to risk contamination of the whole sample).
12. Add 2 ml 100% ethanol dropwise to all tubes while gently vortexing to prevent clustering of the ethanol (the Qiagen columns require ethanol in order to retain the RNA on the column during the initial application and subsequent washing steps).

Qiagen RNeasy purification

The column size is dependant upon the amount of RNA being applied (Check Qiagen kit specifications). This in turn is determined by the initial cell number. When beginning the purification procedure, set the centrifuge temperature to RT.

1. Transfer half of each tube to a Qiagen spin column (the pink top part).
2. Centrifuge up to 5500 rpm and turn off centrifuge once rotor has reached full speed.
3. Filter the second half of the sample through the column.
4. Centrifuge as in step (2) above.
5. Repeat steps 3-4 with filtering the entire sample.

6. Decant liquid from the tube (the RNA should now be retained in the column matrix).
7. Replace the column into the 50 ml tube and fill the column with buffer RW1 (~5 ml is sufficient for a maxi-prep column) for reducing protein contamination.
8. Centrifuge at 5,000 rpm for 5 min.
9. Decant liquid from the tube and repeat steps 7-8.
10. Repeat steps 8-9 twice using ethanol-containing RPE buffer and centrifuging 10 min for the second wash to completely remove all liquid from the column matrix.
11. Place column into a new clean 50 ml tube.
12. Add 500 μ l DEPC-treated water.
13. Sit at room temp for 1 min.
14. Centrifuge at 5,000 rpm for 5 min.
15. DO NOT DISCARD THE LIQUID --- THIS CONTAINS YOUR RNA!!!
16. Repeat steps 14-16 three more times with 10 min spin at the last time.
17. Decant liquid equally into four 1.5 ml eppendorf tubes.
18. Add 2 volumes (~1 ml) of 100% ethanol and mix.
19. Add 40 μ l 3M NaAcetate pH 5.5 (the salt aids in the precipitation of the RNA) and VORTEX!
20. Precipitate RNA at -80°C for 30 min or preferably over night.
21. Centrifuge at 14,000 rpm for a minimum of 30 min at 4°C.
22. Aspirate off supernatant (being careful not to disturb the pellet).
23. Wash the pellet with 500 μ l 75% ethanol by vortexing 10 min.
24. Centrifuge at 14,000 rpm for 15 min and carefully aspirate supernatant.
25. Air dry RNA pellet by open cap for NOT MORE than 30 min (try to place in an RNase-free clean environment for this step).

26. Resuspend the pellet (which should look like a clear gel and may be difficult to see) in DEPC-treated water. Place in the thermo-mixer or water-bath at 65°C for 10 min and then place on ice for 5 min.
27. Quantitate your RNA.
28. Store samples at -80°C