

Isolation of total RNA from tissue and cells

Method

1. For tissues, using pre-chilled long tweezers, place tissue directly into 1 ml of liquid N₂ in a 15-ml, round-bottom tube. Keep the tube at -80°C (without the cap for 24 hr).
Frozen tissue can remain at -80°C indefinitely.
2. Transfer the tissue into a fresh 15-ml, round-bottom tube containing TRI Reagent. We recommend 1 ml TRI Reagent per 50 mg tissue. Increasing the amount of tissue increases the chance of DNA and protein contamination.
3. Immediately homogenize the tissue with Kontes homogenizer and pestle. Be sure that all of tissue is homogenized. Rinse 3X with water between samples.
4. Transfer 750µl of the homogenate into 1.5 ml-microfuge tubes. If thawing homogenates, briefly place in room temperature water bath and swirl.
5. Spin homogenates at 12,000 x g for 10 min. Place supernatant in new microfuge tubes.
- 5a. For cultured cells, remove the culture medium by aspiration, add at least 1 ml TRI Reagent per 10 cm² cells. Pass the cell lysate several times through pipette to complete cell lysis.
6. For tissue and cell lysates, incubate for 5 min at RT.
7. It is beneficial to perform phase separation with bromochloropropane (BCP, MRC # BP 151) instead of chloroform. Use 100 µl of BCP per ml of the reagent. The use of BCP instead of chloroform improves the quality of the isolated RNA. In addition, BCP is less toxic and less volatile than chloroform. If chloroform is used, the volume of

- chloroform should always be 20% of the initial volume of the reagent. Vortex for a full 15 sec and incubate for 3 min at room temperature. Using a higher volume of chloroform will increase DNA contamination.
8. Centrifuge 12,000 x g for 15 min at 4°C.
 9. Transfer aqueous phase to a new centrifuge tube. Do not disturb the interphase.
 10. Add one-half volume isopropanol and incubate samples 10 min at room temperature (not longer, proteins will precipitate). Always add one-half volume isopropanol to the initial volume of the reagent or salts and proteins may co-precipitate.
 11. Pellet RNA 12,000 x g for 10 min at 4°C.
 12. Remove supernatant. Caution: Pellet may be loose. Quickly spin and remove residue.
 13. Add 1 ml 75% Ethanol to pellet, vortex, and centrifuge 7,500 x g for 5 min at 4°C.
 14. Remove Ethanol. Caution: Pellet may be loose. Quickly spin and remove residue with 10- μ l pipette.
 15. Air dry on ice for 5 min (do not dry in hood).
 16. Re-dissolve completely in 87 μ l of RNase-free/low endonuclease water.
 17. DNase/Proteinase K Treatment: Add 10 μ l of DNase buffer, and 3 μ l (6 U) of TURBO DNase (Ambion #2238). Mix and incubate for 15 min at 37°C. Add 4 μ l of Proteinase K (New England BioLabs #P8102S; 20mg/ml). Mix and incubate for 30 min at 37°C.
 18. Use TRI REAGENT LS to extract RNA. Add RNase-free/low endonuclease water to makeup the volume to 0.25 ml.

19. Add 0.75 ml of TRI REAGENT LS and shake vigorously for 15 sec. Store the lysates for 5 min at room temperature.
20. Add 0.1 ml of BCP, cover the sample tightly and shake vigorously. Store the mixture at room temperature for 8 min. Centrifuge the mixture at 12,000 x g for 15 min at 4°C.
21. Transfer the top aqueous phase into a fresh tube. Precipitate the RNA by adding 0.5 ml isopropyl alcohol. Store the samples at room temperature for 8 min and centrifuge at 12,000 x g for 8 min at 4°C.
22. Remove the supernatant and wash 1 ml of 75% ethanol. Centrifuge at 12,000 x g for 8 min at 4°C. Carefully discard the supernatant and air dry the pellet for 5 min.
23. Dissolve the pellet in a small volume of RNase-free/low endonuclease water.
24. For Microarray Analysis the final concentration of RNA should be 2 µg/µl.
25. To determine accurate concentrations and 260/280 absorbance ratios, the RNA should be assayed on a NanoDrop ND-1000 (Wilmington, DE); and to determine quality (intactness), the RNA should be assayed on an Agilent 2100 Bioanalyzer (Foster City, CA).
26. For both the NanoDrop and Agilent 2100 Bioanalyzer analyses, remove 1µg of the RNA sample and dilute to 0.1 µg/µl in 10 µl in TE. The 260/280 absorbance ratio should be above 1.8.

Notes

Precautions when working with RNA:

- Use RNaseAWAY (Molecular BioProducts #7000) for all tubes, bench area, pestles, etc.
- Use nuclease-free water.
- Pre-label all tubes.
- Change gloves often.

For smaller amounts of tissue or numbers of cells, the above protocol can be proportionately scaled down.