TRIzol RNA Extraction

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BACKGROUND

This protocol is used to extract the RNA from tissue or a cell sample using TRIzol and also includes recommendations for 260/280 and 260/230 values when measuring the concentration of extracted RNA using a nanodrop spectrometer.

MATERIALS

- TRIzol reagent
- Chloroform
- Nuclease-free water
- 1.5 mL or 2 mL Eppendorf tubes
- 2 mL VWR Pre-Filled Bead Tubes (if RNA is to be extracted from tissue)
- Container of ice
- Cold PBS
- Isopropanol
- 75% ethanol (use mili Q water for dilution)

EQUIPEMENT

- Forceps
- Homogenizer
- Tabletop centrifuge
- Eppendorf heating plate
- Nanodrop spectrophotometer

PROTOCOL

Before beginning this protocol:

- Prepare a container of ice
- Cool a tabletop centrifuge to 4°C
- Set Eppendorf heating plate to 60 °C
- Turn on fume hood
- Turn on laminar flow hood and UV light for advanced sterility

RNA Extraction from Tissue (Homogenization)

1. Weigh the tissue, make note of the weight (aim for 0.3-0.5 g weight or 10-20 μg dry weight).

***Note: Due to the inhalation hazards associated with TRIzol, steps 2-6 are recommended to be carried out in a fume hood.**

2. Use forceps to remove the tissue from container and place into new 2 mL VMR pre-filled bead homogenization tube with 900 μL of TRIzol reagent.
3. Homogenize the tissue using a Fisherbrand Bead Mill 24 Homogenizer at 4.5 m/s, 10 s × 2 cycles, 10 s dwell, or until there are no visible pieces of tissue. If homogenizing multiple samples at once, more time may be required.
4. Move lysate into a new 2 mL tube and centrifuge at 12,000 g at 4°C for 10 minutes.
5. Transfer the supernatant to a new 2 mL tube and avoid disrupting the pellet.
    a. Roughly 700 μL TRIzol will be recovered
    b. Pellet = cell debris, beads
    c. Top clear layer = lipids (more obvious in fatty tissues)
    d. Pink solution = Nucleic acids
6. Mix by pipetting.

RNA Extraction from Cells (Separation from Media)

1. (If cells are suspended in RNAsave) add an equal volume of cold PBS to the RNAsave to dilute.
    a. RNAsave will be too heavy for the cells to centrifuge out of otherwise.
2. Centrifuge sample at 500 g for 5 minutes.
3. Discard the supernatant.

***Note: Due to the inhalation hazards associated with TRIzol, steps 4-5 are recommended to be carried out in a fume hood.**

4. Resuspend the cells in 700 μL TRIzol to the sample.
5. Mix by pipetting.

Common RNA Extraction

***Note: Due to the inhalation hazards associated with TRIzol and chloroform, steps 1-3 are recommended to be carried out in a fume hood.**

1. Incubate the TRIzol containing the sample at room temperature for 5 minutes after removing from centrifuge.
    a. This step is necessary to allow the nucleoprotein complex to dissociate.
2. Add 300 µL chloroform to the TRIzol containing the solution.
3. Securely cap the tube, then thoroughly mix by shaking for approximately ~15 seconds.
4. Incubate for 3 minutes at room temperature.
5. Centrifuge the sample at 12,000 g at 4°C for 15 minutes.
6. Transfer the aqueous phase (top clear layer) containing the RNA to a new 1.5 mL tube:
    a. Angle the tube at 45° and pipette the solution out to avoid touching the interphase or organic layer.
    b. Roughly 400 µL aqueous phase solution will be recovered.
7. Add 500 µL isopropanol to the aqueous phase.
8. Invert samples to mix.
9. Incubate at 4°C (on ice) for 10 minutes.
10. Centrifuge at 12,000 g at 4°C for 10 minutes.
    a. RNA precipitate should form white gel-like layer at bottom of tube – the pellet may be very faint at this point, but it should become clearer over subsequent washes.
11. Now working in a laminar flow hood, discard the supernatant by carefully pipetting (set aside in case RNA extraction is not concentrated enough and a re-wash is required).
12. Resuspend RNA in 700 µL 75% ethanol.
    a. The 25% H_2O will pull out water soluble salts, solubilize proteins.
13. Mix by flicking and inverting.
14. Centrifuge at 12,000 g at 4°C for 5 minutes.
15. Discard the supernatant.
    a. Supernatant removal can be improved by removing any remaining supernatant with a p1000 then a p200, especially for the final supernatant.
16. Repeat steps 11-15 twice more (for a total of 3 washes).
17. Air-dry the RNA pellet in tube by leaving the tube with the cap open for 10 minutes or 45 minutes if the RNA is extracted from tissue under the laminar flow hood with the fan on. If the ethanol has not evaporated in 10 or 45 minutes, let it dry for an additional 5-10 minutes.
18. Resuspend the pellet in 20–50 µL of RNase-free water by gently pipetting up and down.
    a. Opt for 20 µL if unsure hoe concentrated the RNA is (RNA can be diluted later).
19. Incubate at 60 °C for 10 minutes.

20. Measure the concentration of RNA using a nanodrop spectrophotometer.
    a. Minimum RNA values:
        i. 260/280 → >1.70
            1. Low value suggests protein contamination → re-extract.
        ii. 260/230 → >2
            1. Low value suggests salt/chemical contamination —> re-wash (repeat steps 11-18).
    b. Note that measuring samples by nanodrop consumes 2 µL – be sure to take this into account when diluting and preparing enough sample for subsequent experiments.
21. Store the extracted RNA solution in a -80°C freezer or immediately dilute and use the RNA for cDNA synthesis.

NOTES

- https://assets.thermofisher.com/TFS-Assets/LSG/manuals/trizol_reagent.pdf
- See above use manual for further information.
- After step 4, TRIzol RNA extraction will separate the sample into a lower red phenol chloroform, an interphase, and a colorless upper aqueous phase:
    - Aqueous phase → RNA
    - Interphase & organic phase → DNA & protein