

Total RNA Isolation

This protocol was developed for use with easily disrupted tissues such as brain or liver and with cells from tissue culture. More fibrous tissues may require additional treatment steps in order to obtain full purity.

Important! Work in an RNase FREE area and work on ice unless otherwise indicated. Use RNase-free H₂O, tubes and pipettes.

1. Incubate homogenizer in 3% peroxide for 10 min prior to use. Wash with RNase-free H₂O. Clean the homogenizer with RNase Zap between samples.
2. Place frozen mouse brain tissue, ~100 mg, in 2 ml TRIzol Reagent (1 ml of TRIzol Reagent per 50-100 mg of tissue).
3. Homogenize tissue at moderate to high speed for 1 min.
4. Incubate the homogenized sample for 5 min at R/T.
5. Add 0.2 ml of Chloroform per ml of TRIzol Reagent. Cap tubes securely.
6. Handshake for 15 sec and incubate for 2-3 min at R/T.
7. Transfer aliquots of 500 μ l (up to 750 μ l) homogenates to a pre-spun (12,000 rpm for 30 sec) yellow heavy PLG I tube (Phase Lock Gel tube I).
8. Centrifuge for 10 min at 12,000 rpm at 4°C.
9. Remove upper colorless aqueous phase remaining the RNA to a fresh tube.
10. Precipitate RNA with 0.5 ml isopropanol per ml of TRIzol Reagent.
11. Incubate for 10 min at R/T.
12. Centrifuge at 12,000 rpm for 10 min at 4°C.
13. Remove the supernatant carefully.
14. Wash with 1 ml 75% ethanol per ml TRIzol Reagent.
15. Vortex and centrifuge at 9,000 rpm for 5 min at 4°C.
16. Remove the supernatant and briefly dry the RNA pellet by air-dry for 5-15 min or vacuum-dry. Do not dry RNA by centrifugation under vacuum. It's very important that the RNA pellet does not dry completely.
17. Dissolve RNA in 30 μ l RNase-free water by passing the solution a few times through a pipette tip, and incubating for 10 min at 55-60°C.
18. Take an aliquot of 1 μ l or more for quality and quantity measurement. The expected yield of RNA per mg brain tissue is 1-1.5 μ g.
19. Store sample at -80°C if necessary, or go on to next step.

Cleanup of total RNA (RNeasy Mini Kit)

Important! Do not exceed 100 μ g RNA/spin column. Add 10 μ l β -ME per ml of RLT buffer. Make sure 4 volumes 100% ethanol was added to the RPE buffer. All centrifugation steps should be performed at 20-25°C; max speed = ~8,000g (14,000 rpm).

1. Adjust the volume of the total RNA sample to 100 μ l with RNase-free water.
2. Add 350 μ l buffer RLT (with β -ME) to the sample and mix thoroughly.
3. Add 250 μ l 100% ethanol to the lysate and mix by pipetting. Do not centrifuge!

4. Apply sample (700 μ l) to an RNeasy column sitting in a 2ml collection tube. Spin for 15 sec at max speed. Discard the flow-through.
5. Add 500 μ l RPE buffer (ethanol added) onto the column and spin for 15 sec at max speed to wash. Discard the flow-through.
6. Add an additional 500 μ l RPE buffer and spin at max speed for 2 min to dry the RNeasy membrane.
7. Carefully, without touching the ethanol, transfer column to a new 1.5ml tube and pipette 30-50 μ l of RNase-free water directly onto the membrane. Wait for 3-4 min! Spin at max speed for 1 min to elute.
8. Repeat step 7 if more than 30 μ g RNA yield is expected and elute into the same collection tube.
9. Take an aliquot of 1 μ l or more for quality and quantity measurement if necessary.

Precipitation of RNA

1. Add 1/10 volume 3 M NaAcetate, pH 5.2, and 2.5 volumes 100% ethanol.
2. Mix and incubate at -20°C for at least 1 hour or over-night.
3. Centrifuge at max speed in a microcentrifuge for 30 min at 4°C .
4. Wash pellet twice with 80% ethanol, and spin for 5 min at max speed at 4°C .
5. Air-dry pellet. Check for dryness before proceeding.
6. Resuspend pellet in DEPC-treated H₂O. The appropriate volume for resuspension depends on the expected yield and the amount of RNA required for the cDNA synthesis.
7. Take 1 μ l or an aliquot for quality and quantity measurement.

Total RNA quality and quantity

1. **Alt. 1.** Check the total RNA quality on 1% agarose (RNase free) gel by loading 1 μ l of the total RNA sample. Treat gel equipment with RNase Zap before use. Use RNase free water to make TAE buffer needed for the agarose gel preparation and electrophoresis buffer. Run at 60 V for 30 min. Look for 2 kbp (28S), 0.9 kbp (18S), and 200bp (5S) ribosomal RNA bands.
Alt. 2. Check the total RNA quality using the Agilent 2100 BioAnalyzer. Use the assay for total RNA. The electropherogram should exhibit two ribosomal peaks only with a low background level of signal from mRNA.
2. Add 1 μ l of the total RNA sample to 9 μ l of 1 x TE to quantify the total RNA. The absorbency should be checked at 260 nm and 280 nm for determination of sample concentration and purity. 1 OD at 260 nm equals 40 μ g RNA per ml. The A₂₆₀/A₂₈₀ ratio should be between 1.9-2.1 for pure RNA when RNA sample is diluted in 10mM Tris-Cl, pH 7.5.