Sep 06, 2024

# RNA Isolation with TRIzol™ (Invitrogen) and on column DNase treatment using Qiagen RNeasy

DOI
**dx.doi.org/10.17504/protocols.io.6qpvr8ynzlmk/v1**

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**DOI: dx.doi.org/10.17504/protocols.io.6qpvr8ynzlmk/v1**

**Protocol Citation:** David Metzger 2024. RNA Isolation with TRIzol™ (Invitrogen) and on column DNase treatment using Qiagen RNeasy. **protocols.io https://dx.doi.org/10.17504/protocols.io.6qpvr8ynzlmk/v1**



**Protocol status:** Working
**We use this protocol and it's working**

**Created:** September 06, 2024

**Last Modified:** September 06, 2024

**Protocol Integer ID:** 107034

## Abstract

This protocol is a compilation of the protocols for isolating total RNA from frozen tissue using TRIzol (Invitrogen) and DNase treating total RNA using the Qiagen RNA clean up protocol. The text and workflow have been largely taken and adapted from the manufacturers standard protocols from Invitrogen and Qiagen. The resulting RNA is suitable for most downstream applications including RNA-seq and qRT-PCR.

Original manufacturer protocols can be found at:
TRIzol (Invitrogen): https://assets.thermofisher.com/TFS-Assets/LSG/manuals/trizol_reagent.pdf
Qiagen RNeasy: https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/rna-purification/total-rna/rneasy-kits

## Homogenization with NextAdvanced Bullet Blender

1 Place ~10 1.0 mm ceria stabilized zirconium oxide beads in a safe-lock tube for each sample.

2 In a chemical fume hood, add 1 mL of TRIzol reagent to the Safe-Lock tube containing the beads.

3 Remove frozen tissue from -80°C and put directly into the tubes containing TRIzol.

Note:The optimum sample size for 1 mL of TRIzol is between 50-100 mg; if over 100 mg, adjust the volume of TRIzol accordinly and split the sample into two pieces and homogenize in separate tubes. Thse can be combined afterhomogenization.

4 Place samples in the Bullet Blender homogenizer. Homogenize for 30s to 1min on setting 8. Repeat this step as many times as needed until the tissue is fully homogenized. Incubate samples on ice between each homogenization cycle.

Notes:
The number of homogenization cycles will depend the size of the tissue and the tissue type. If possible, homogenize some test samples first to identify the most appropriate homogenization prodedure.

Leave at least three empty spaces between tubes in the homogenizing. Fully loading the bullet blender will reduce the effectiveness of the homogenizer and this step will take much longer.

Using the homogenizer on full speed (9 or 10) will risk having the sample tubes pop oven and spilling TRIzol. This not only creates a safety hazard but also results in the loss of precious samples.

Homogenized samples can be stored at -80C for future extraction.

## Phase Separation

5 Incubate homogenized samples for 5min at room temperature to allow complete dissociation of the
nucleoproteins complex.

6 In a chemical fume hood, add 0.2 mL of chloroform per 1 mL of TRIzol™ .

Securely cap the tube, then thoroughly mix by shaking vigorously by hand for 15s or by using a vortex on a medium setting and vortexing the samples on their side.

7 Incubate the samples at room temperature for 3 minutes.

8 Centrifuge the sample for 15 minutes at 12,000 × g at 4°C.

9 Following centrifugation, the mixture separates into a lower red phenolchloroform phase, an interphase and a colourless, upper aqueous phase. RNA remains exclusively in the colourless aqueous phase (which is about 60% of thevolume of TRIzol reagent used for initial homogenization).

10 Using a p1000, carefully transfer the aqueous phase containing the RNA to a new tube by angling the tube at 45° and pipetting the solution out.

Note: Only pipette ~0.5ml of the aqueous phase. It is better to leave some of the aqueous phase behind to reduce the amount of DNA and organic material from the other phases.

## RNA Precipitation

11 Add 0.5mL of isopropanol per 1mL of TRIzol regent originally used during homogenization, to the tube containing the colourless aqueous phase.

12 Allow the RNA to precipitate by incubating the samples for 10 minutes at 4°C.

13 Pellet the precipitated RNA by centrifugation for 10 minutes at 12,000 × g at 4°C.

Note: The RNA precipitate will from a white gel-like pellet on the side and bottom of the tube. When loading your tubes in the centrifuge, position the hinge facing up. The pellet will form on the hinge side at the base of the tube. This will help with the RNA wash in the next section.

## RNA Wash

14 Carefully remove the supernatant being careful not the disrupt the RNA pellet. Use a p1000 to remove most of the supernatant and a p20 or p200 to remove the rest.

15 Wash the RNA pellet with 1 mL of 75% ethanol per 1 mL of TRIzol Reagent used for lysis. Briefly mix by inversion or a very quick pulse vortex to dislodge the pellet. This will ensure the RNA pellet is completely washed by the ethanol.

16 Centrifuge for 5 minutes at 7500 × g at 4°C.

## Solubilize the RNA

17 Remove supernatant with a pipet. Quickly spin in a centrifuge to collect any remaining 75% ethanol to the bottom. Remove as much of the remaining ethanol with a pipet and air dry the RNA pellet by leaving the
tubes open on the counter for approximately 15-30 minutes. When the pellet is dry, there must be no visible ethanol in the tube. Do not over dry the pellet as it may be difficult to redissolve.

18 Resuspend the RNA pellet in 20-50 µL of nuclease-free water by pipetting up and down gently. Store RNA at -80°C for future use.

NOTE: If the pellet does not go into solution, briefly incubate the sample Incubate the sample at 55–60°C for a several minutes. Check the sample frequently and place on ice once the pellet has completely solubilized. Flick gently to mix and quickly spin to collect the solution.

## DNase treatment using QIAGEN RNeasy spin columns

19 For downstream applications of RNA it is often necessary to remove any DNA contamination from the total RNA sample obtained from the TRIzol RNA isolation. This can be quickly and effectively done using QIAGEN RNeasy spin columns.

20 Prepare DNase I stock solution before using the RNAse-Free DNase Set for the first time. Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNAse-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNAse-free water into the vial using an RNAse-free needle and syringe. Mix gently by inverting the vial. Do not vortex.

For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at −30 to −15°C °C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

21 Adjust the sample to a volume of 100 µl with RNase-free water.

22 Add 250 µl 100% ethanol to the diluted RNA, and mix well by pipetting.

23 Transfer the sample (700 µl) to an RNeasy Mini spin column placed in a 2 ml collection tube (supplied). Close the lid. Centrifuge for 15 s at ≥8000 x g. Discard the flow-through.

24 Add 350 µl Buffer RW1 to RNeasy column, close lid, centrifuge for 15 s at ≥8000 x g (≥10,000 rpm). Discard flow-through.

25 Add 10 µl DNase I stock solution (see above) to 70 µl Buffer RDD. Mix by gently inverting the tube. Centrifuge briefly.

Note: I typically make a mastermix for the total number of samples that I'm processing.

26 Add DNase I incubation mix (80 µl) directly to RNeasy column membrane, and place on benchtop (20–30°C) for 15 min.

27 Add 350 µl Buffer RW1 to RNeasy column, close lid, centrifuge for 15 s at ≥8000 x g. Discard flow-through.

28 Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid. Centrifuge for 2 min at ≥8000 x g to wash the membrane.

Optional: Place the RNeasy spin column in a new 2 ml collection tube (supplied). Close the lid, and centrifuge at full speed for 1 min

29 Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid, and centrifuge for 1 min at ≥8000 x g to elute the RNA.

Note: To increase the RNA recovery from the column, this step can be repeated again using another 30–50 µl of RNase-free water. Alternatively, use the original eluate if high RNA concentration is required and reuse the collection tube. I typically perform this second option.