Jul 15, 2025

# RNA extraction from Tigriopus californicus (splashpool copepods) - optimized in Spring 2024 by Rujuta Vaidya and Isabelle Neylan

DOI
https://dx.doi.org/10.17504/protocols.io.yxmvmbx3ng3p/v1

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OPEN ACCESS

**DOI:** https://dx.doi.org/10.17504/protocols.io.yxmvmbx3ng3p/v1

**Protocol Citation:** Isabelle Neylan, Rujuta Vaidya 2025. RNA extraction from Tigriopus californicus (splashpool copepods) - optimized in Spring 2024 by Rujuta Vaidya and Isabelle Neylan. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvmbx3ng3p/v1

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

**Created:** July 15, 2025

**Last Modified:** July 15, 2025

**Protocol Integer ID:** 222484

**Keywords:** rna extraction from tigriopus californicus, rna extraction, rna, splashpool copepod, sufficient rna yield, high quality rna molecule, tigriopus californicus, extraction, quality rna

## Abstract

A combination of Trizol reagent and Qiagen kit gives the sufficient RNA yield and high quality RNA molecules (no shearing, smaller bands on the gel)

## Guidelines

4. Add 1000 uL of TRIZOL reagent to each tube - total amount of TRIZOL reagent needed per sample is 1000uL, but adding it all at once makes it splash as you are crushing animals.

5. Incubate the tubes at room temperature for 5 minutes.

6. Add 200uL of chloroform to each tube, and shake thoroughly (as if your life depends on it) to mix the contents for a couple of minutes). The solution in the tubes should turn a light pink shade (like pepto bismol pink).

7. Incubate at room temp for 3 minutes.

8. Centrifuge at 4 degree celsius for 15 minutes at 13000 rpm.

9. The solution in the tube has now separated into three layers - DNA is in interphase between the lower pink layer and upper aqueous layer of RNA. We want to separate this upper RNA layer without taking in any DNA or protein. DNA tends to stick to the side of the tubes as a thin fatty layer.

10. Take one tube out of the centrifuge while maintaining its tilted 45 degree angle, and gently pipette out the upper, transparent layer that is RNA on a Qiagen gDNA eliminator spin column placed in a 2 mL collection tube. Use 200uL pipette and be careful not to touch the sides of the tube while pipetting RNA layer. Get as close to the pink layer as you can without disturbing it. We usually get around 300 -400 uL worth of RNA layer. Work your way through samples one tube at a time (set pipette to 180uL and usually takes 2-2.5 pulls ~ 360 to 400 uL).

**ALL centrifugation steps from step 11 onwards happen at room temperature.**

11. Centrifuge gDNA columns for 30 seconds (s) at 10,000 rpm. Discard the column, and save the flow-through.

12. Add 1 volume of 70% ethanol to the flow-through, and mix well by pipetting. Do not centrifuge. (typically 380 uL for our samples)

13. Transfer up to 700 uL of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 30 s at 10,000 rpm. Discard the flow-through. Reuse the collection tube. If the sample volume exceeds 700 uL, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation. (**happens twice to get all of the liquid through the column, which is always more than 700uL**)

14. Add 700 uL Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 30 s at 10,000 rpm to wash the spin column membrane. Discard the flowthrough.

Reuse the collection tube.

*Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.*

15. Add 500 uL Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 30 s at 10,000 rpm to wash the spin column membrane. Discard the flowthrough. Reuse the collection tube.

*Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Important points before starting").*

16. Add 500 uL Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min **10,000 rpm** to wash the spin column membrane. The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.
*Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.*

17. Place the RNeasy spin column in a new 2 ml collection tube (supplied), and discard the old collection tube with the flow-through. Centrifuge at full speed for 1 min. Perform this step to eliminate any possible carryover of Buffer RPE, or if residual flow-through remains on the outside of the RNeasy spin column.

18. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 40 uL RNase-free water directly to the spin column membrane, **incubate at room temp for 5 mins**. Close the lid gently, and centrifuge for 1 min at 10,000 rpm to elute the RNA.

19. Take out tubes from the centrifuge and immediately place them on ice. **Extracted RNA should always be in -80 freezer or on ice when in use for reactions/analysis, never left unattended at ROOM TEMPERATURE.** Repeated freeze thaws degrade RNA quality.

20. Aliquot 1uL of freshly extracted RNA in PCR strip tubes if you are planning on sending it for RIN analysis on 6th floor, before you store your RNA samples in -80 freezer - avoid thawing frozen RNA at all costs.

## Materials

Trizol reagent, Qiagen kit, pestles, benchtops, pipettes, RNAase Away spray, 70% ethanol, kimwipes, aliquoting buffer RPE1, 50mL falcon tube, centrifuge, drill bits, gloves, icebox, RLT buffer, tissue ruptor, chloroform, 2 mL collection tubes, 200uL pipette, Qiagen gDNA eliminator spin column, 70% ethanol, RNeasy spin column, Buffer RW1.

## Before start

Pestles, benchtops, pipettes should be wiped with RNAase Away spray. Pestles should be cleaned with 70% ethanol and kimwipes first. Aliquot buffer RPE1 in 50mL falcon tube as per Morgan's suggestion to reduce ethanol loss during daily usage. The centrifuge needs 10-ish minutes to get to 4 degree celsius. Clean blender tips/drill bits with ethanol and RNAseaway. Wear gloves at all times when handling Trizol. Do not get too close to the giant chloroform bottle, especially if working alone on weekends. Prep everything before taking samples out of -80 freezer. Samples should either be in -80 freezer or in Trizol reagent, not hanging around somewhere in between. Process things in smaller batches - no more than 3 to 6 tubes per individual per round of extractions.

1 Get frozen samples out of -80 and place them in your icebox—take your ice box to the freezer and add them one at a time. Sometimes the tubes shoot off—that's just life.

2 Add 50uL of RLT buffer to each sample tube immediately and then begin crushing the frozen copepods. The crushing does not need to happen on ice, as long as the copepods are submerged in TRIZOL. Be quick about crushing, and as you move through tubes, make sure they are kept on ice while you work your way through samples. (we changed this because the trizol reagent was melting our tissue ruptor blender tips and making them unusable for the next round of extractions).

3 Grind the copepods with tissue ruptor (takes 30 to 60 seconds) until you do not see any intact copepods floating around. A pulpy-looking liquid is ideal.

4 Add 1000 uL of TRIZOL reagent to each tube - total amount of TRIZOL reagent needed per sample is 1000uL, but adding it all at once makes it splash as you are crushing animals.

5 Incubate the tubes at room temperature for 5 minutes.

6 Add 200uL of chloroform to each tube, and shake thoroughly (as if your life depends on it) to mix the contents for a couple of minutes. The solution in the tubes should turn a light pink shade (like pepto bismol pink).

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