

RNA extraction for Lipid-Rich tissues (Zymo kit)

Purpose:

To extract high-quality RNA from homogenized tissues, especially lipid-rich tissues such as hypothalamii, brain, thymus, or crop.

Reagents:

- Zymo Direct-Zol RNA mini kit (Genesee)
- TriSure reagent (BioLine; stored at 4 C) OR TRIzol reagent (ThermoFisher)
- Chloroform (flammables cabinet, hood)
- 21 G needles and 1 mL syringes (1 per sample)
- 100 percent Ethanol (flammables cabinet)
- 1.7 mL tubes for storage of extracted RNAs

Instructions:

1. Homogenize tissue in 750 uL TriSure per **10 mg** of sample (or less).
(*See Tissue Homogenization protocol*)

Note: Brain tissue will get foamy during homogenizing. Don't let the foam get out of the tube!

Note: With help, move the centrifuge into the bottom shelf of the fridge to cool it for later spin steps.
2. Add 250 uL additional TriSure to homogenized sample in solution.
3. **Second homogenization:** Pass entire sample 10 times through clean 21 gauge (26 gauge also worked) needle, attached to a 1 mL syringe. Store used syringes in the "dirty" bag, throw used needles in the sharps waste.
4. Let homogenate sit at room temperature for 5 minutes to allow nucleoprotein complexes to dissociate.

5. While waiting for above step, **PUT BACK any tissues that are still out in -80 C freezer A.S.A.P!!**

6. In the hood, pour out only enough chloroform for 200 uL per sample into a large 10 mL falcon tube. (e.g. 2 -3 mL for 10 samples). *Chloroform evaporates quickly so we don't want to pour more than is necessary.*

7. In the hood, add 200 uL chloroform to each sample. Vortex vigorously for 15 seconds in the hood.

8. Let homogenate sit at room temperature for 5 minutes in the hood.

Note: Begin thawing DNase I mixture on ice or in fridge after you finish homogenization. DNase I stored in the RNA extraction box in the -20 C freezer.

9. **Phase separation spin:** In the fridge (4 C), centrifuge samples for 15 minutes at 12,000 rpm.

Note: This is a good time to prepare numbered 2 mL tubes and spin columns/collection tubes for each sample.

10. Transfer upper aqueous phase (about 600 uL) to a new collection tube (these are the same 2 mL tubes used in during tissue homogenization). *Take care not to get any of the green-turquoise layer into the tube.*

Do NOT pour out the other phases. Save the remaining phases in the original tubes in the - 20 C freezer as organic phase. We use this to extract DNA or protein later, if desired.

Note how much the aqueous phase is, you will need to add this volume of ethanol in the next step. For example, if your sample only had 500 uL of aqueous phase transferred, only add 500 uL of EtOH in the next step.

11. Add 1 volume (e.g. 600 uL in previous step) of 100 percent EtOH to transferred aqueous phase. Mix by pipetting up and down, or vortex at a *low* setting.

The following steps are the same as steps 3 - 12 of the regular Zymo RNA extraction protocol.

12. Load 700 uL EtOH-sample mixture into column (= filter + tube from yellow Zymo box)). Spin for 1 minute at 12,000 rpm. Pour out flow-through.

Repeat until all of the mixture has been loaded onto the column and spun.

13. Wash column with 400 uL RNA wash buffer before DNA digest step. Centrifuge for 30 sec at 12K rpm. Pour out flow-through.

14. **DNase treatment:**

Prepare the DNase-buffer mix by mixing the following volumes of DNase buffer and enzyme into a new tube:

DNase buffer = $75 \times 1.05 \times \text{number of samples}$ uL

DNase enzyme = $5 \times 1.05 \times \text{number of samples}$ uL

Load 80 uL of the DNase-buffer mix directly onto the column of each sample. Gently touch tip to filter, use a different tip each time.

Incubate samples at room temperature for 15 minutes.

Note: Now is a good time to prepare labeled final 1.7 mL tubes for each sample. Put back DNase in freezer.

After incubation, centrifuge for 30 sec at 12K rpm. Pour out any flow-through.

15. **Prewash:** Add 400 uL pre-wash buffer and centrifuge samples for 1 minute at 12K rpm. Pour out flow-through.
16. Repeat step 15.
17. **Wash column:** Add 700 uL RNA Wash Buffer (green cap) to column directly, touching filter gently and using a different tip for each sample. Spin for 1 minute at 12K rpm. Pour out flow-through.
18. Spin empty columns for another 1 min at 12K rpm to remove all excess wash buffer.
19. **Elution:** Place columns in their final 1.5 mL storage tubes (Final tubes need to be labeled on top and side with sample ID, RNA, and date.)

Add 20 uL DNA/RNase free H₂O to each column. Centrifuge for 1 minute at 12K rpm. Let sit at room temperature for 1 minute.
20. Repeat step 19 for a total elution of 40 uL.
21. Nanodrop immediately, or store in a labeled rack or box in the -20 C freezer.

Check one last time to be sure any tissues/dry ice have been put away in the -80 C freezer. **MAKE SURE RNAs ARE IN THE FREEZER!**

Source protocol:

Modified from the laboratory of Dr. Christopher Mason "Extract Total RNA from Lipid Tissues" protocol, Cornell University, 2011.

Accessed from: http://physiology.med.cornell.edu/faculty/mason/lab/zumbo/zumbos_documents/DOCUMENTS_files/ZUMBO_rna_isolation_tissues.pdf

Updates:

Protocol written 7/31/17 by V. Farrar

Version 1.2: Updated to reflect notes by R.Viernes (2018-19)

Version 1.3: Small updates by V. Farrar (10/29/2019)

Version 1.4: Updates for brain nuclei by V.Farrar (2021-02-19)

Version 1.5: Indicate saving organic phase for downstream applications.
(2021-05-15)