RNAzol RT/Direct-zol RNA extraction and purification protocol (total RNA)

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Protocols:

<https://www.mrcgene.com/wp-content/uploads/2017/04/RNAzolRTMarch2017.pdf>

<https://files.zymoresearch.com/protocols/_r2060_r2061_r2062_r2063_direct-zol_rna_microprep.pdf>

(Before start) Prepare DNase:

5 ul DNase I (6 U/ul), 35 ul DNA digestion buffer – amount for each sample

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1. Cut 15 mL falcon tubes in half at the 10 mL mark, put bottom halves (without cap) on ice
2. Aliquot 2.5 mL of RNAzol RT into the tubes and store on ice.
3. Blot oyster tissue as dry as possible on brown paper towel
4. Transfer whole oyster body to tubes containing RNAzol RT.
5. Homogenize immediately with disposable pestle.
6. Transfer to intact 15 mL falcon tubes, vortex 15s.
7. Add 1.2 mL of 0.1% DEPC-treated H2O to tubes.
8. Vortex 15s.
9. Incubate at room temperature (RT) for 15mins.
10. Centrifuge 9,500 RPM on the large centrifuge with the SL-50 rotor (in the glass front fridge in 213) for 15mins @ RT.
11. Transfer 750uL of supernatant (do not disturb pellet) to sterile 1.7mL snap-cap tube. (Keep remaining sample on ice and eventually move to the box in the -80 for our bigger samples.)
12. If the supernatant is cloudy, spin it down even more in the snap-cap tube (10,000g for 3-5 min), and transfer THAT supernatant to a new tube. Supernatant shouldn’t be cloudy or murky at all.
13. Add 750uL of 2-propanol (isopropanol) or 100% ethanol to fresh supernatant.
14. Vortex 5s.
15. Incubate @ RT for 5mins.
16. Transfer mixture into Zymo-Spin IC column w/ collection tube
17. Centrifuge 10,000g for 30s (or until all cleared)
18. Transfer column into a new collection tube, discard flow-through
19. Add 400uL **RNA Wash Buffer** to column
20. Centrifuge 10,000g for 30s
21. Add 40uL of DNase solution directly to column
22. Incubate @ RT for 15mins
23. Add 400uL Direct-zol RNA PreWash to column
24. Centrifuge 10,000g for 30s
25. Discard flow-through. Repeat steps 21-22.
26. Discard flow-through. Add 700ul **RNA Wash Buffer**.
27. Centrifuge 10,000g for 1 min
28. Transfer column to RNase-free tube
29. Add 50uL of DNase/RNase-Free water
30. Centrifuge 10,000g for 30 sec.
31. Keep sample on ice for short-term storage (i.e., no more than 2hrs); Store @ -80C. Do Qubit and nanodrop analyses if there’s time.

**Nanodrop analysis**

1. Open the lab computer in the back on the “srlab” account and type in the password “Scallop”. If the computer’s already logged in, skip this step.
2. Open the “Nanodrop” application on the left side of the screen. It has a slightly different name, but I can’t remember. Choose the “nucleic acids” option when the window first opens.
3. It will ask you to put water on it for a blank. Carefully pipette 1 uL of DEPC treated water (molecular grade water) onto the the black spot on the nanodrop, put the top bar down, and press okay. This calibrates the nanodrop for your samples.
4. Change the setting on the top right to “RNA-40” from “DNA.” Use the water you just blanked the machine with to do another blank for the RNA setting, and press “blank” in the top left corner of the nanodrop window.
5. Wipe off the spot vigorously with a kimwipe, and put 1 uL of your first sample onto the black spot. Make sure it’s directly on the spot, with no air bubbles or anything else. Put the top bar down and press “measure” in the top left of the window.
6. Record the 260/280 and 260/230 values in the Google sheet. Anything in the range of 1.8 to 2.0 for both of them is good! Also, if the curve looks like anything other than below, make a note of it in the google sheet. Like the graph below says, salts will show up at lower wavelengths (which means we need to go through the wash step again with our sample), and proteins will be further up, which may mean the original sample is contaminated or wasn’t washed enough. **If nanodrop analyses look good for some or all of the samples, move onto the Qubit.**

A picture containing plot, text, line, screenshot

Description automatically generated

**Qubit analysis**

1. Take out the little tubes from the top drawer below the Qubit, the RNA broad range (“BR”) dye, and the RNA BR buffer. Label the little tubes with your sample ID’s **on the top cap**. If you label it on the side, the Qubit won’t be able to read the tube. Make sure to only use the samples that passed the nanodrop.
2. Get a microcentrifuge tube (the same type the samples are stored in), and put in 199 uL of buffer and 1 uL of dye per sample you have plus an extra. For example, if you have five samples, put in 199\*6 = 1,194 uL of the buffer, and 1 \*6 = 6 uL of the dye. Vortex this briefly.
3. Put 199 uL of this mixture into each little tube. Then, put 1 uL of your corresponding sample into its appropriate, labeled tube.
4. Touch the Qubit screen to fire it up. Click on the “RNA” option. Select the “RNA broad range” option. Put one of your tubes in the top of the machine and press “read tube.” Repeat for every sample, noting the concentrations in the Google sheet as you go.