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Extract dinoflagellates (Karenia brevis) RNA from filter samples. V.1

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We use this protocol and it's working

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Abstract

This protocol is designed for the extraction of total RNA from filter samples containing phytoplankton. It has been validated with a total of 10^5 cells. The entire procedure requires approximately 2 hours to complete and can yield a minimum of 2µg of RNA, varying based on the sample concentration. The RNA Integrity Number (RIN) obtained should be above 7.

Materials

RNeasy Plant Mini Kit Qiagen
RNase-Free DNase Set Qiagen

Before start

- Add 10 µl β-mercaptoethanol (β-ME) to 1 ml Buffer RLT or Buffer RLC before use. Buffers with DTT or β-ME can be stored at room temperature for up to 1 month.
- Add 4 volumes of ethanol (96–100%) to Buffer RPE for a working solution.
- Dissolve lyophilized DNase I (1500 Kunitz units) in 550 µl of RNase-free water. To avoid loss, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting. Do not vortex.
- For long-term storage of DNase I, remove the stock solution from the glass vial and divide it into single-use aliquots. Aliquots can be stored at –15 to –25°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. **Note:** Do not freeze the aliquots after thawing. Do not vortex the reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube.

1 Retrieve the 2 mL Cryovial tube holding the sample from the -80°C freezer, and immediately transfer it to dry ice contained within an ice box for temperature preservation.

2 Add 450 mL RTL buffer to a sterile 2 mL Eppendorf tube with three (ea) 2.8 mm ceramic beads.

3 Using tweezers, securely hold the filter. Next, finely cut it using scissors. Carefully add the cut pieces into the 2 mL tubes from step 2.

4 Spin the tubes for 00:00:10 in a mini-centrifuge to ensure all filter pieces are fully immersed in the RLT buffer. 10s

5 Place the tubes on dry ice for 00:05:00 (or until frozen) and then thaw quickly (slightly warmer than RT). 5m

6 Immediately, after thawing, disrupt the filters using a TissueLyser for 00:05:00 at the maximum speed (30/s). **Note:** Wrap the cap of the tube with parafilm to prevent any leakage of lysates. 5m

7 Spin the tubes for 00:00:10 to ensure all filter pieces are fully immersed in the lysates. Leave the tubes for 00:02:00 at room temperature. 2m 10s

8 Transfer the lysate and filters to a QIAshredder spin column (lilac) placed in a 2 mL collection tube. centrifuge for 13.000 rpm, 00:02:00. Transfer the supernatant of the flow-through to a new 2 mL microcentrifuge tube (not supplied) without disturbing the cell-debris pellet. 2m

9 Add 0.5 volume of Ethanol (96–100%) to the cleared lysate, and mix immediately by pipetting. Do not centrifuge. Proceed immediately to step 10.

10 Transfer the sample (usually 650 µl), with any precipitate, to an RNeasy Mini spin column (pink) in a 2 mL collection tube (supplied). Centrifuge for 10000 rpm, 00:00:30. Discard the collection tubes and replace them with new ones. 30s
Note: Don't load more than 700 µL on the column. If the sample is more than 700 µL, repeat this step by transferring the rest to the spin column and centrifuge again.

11 Add 350 µL Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 10000 rpm, 00:00:30 to wash the spin column membrane. Discard the flow-through. 30s

12 Add 10 µL DNase I stock solution (see before start) to 70 µL Buffer RDD. Mix by gently inverting the tube. Centrifuge briefly to collect residual liquid from the sides of the tube. Buffer RDD is supplied with the RNAse-Free DNase Set.

13 Add the DNase I incubation mix (80 µL) directly to the RNeasy spin column membrane, and place on the benchtop at Room temperature for 00:15:00 . 15m

14 Add 350 µL Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 10000 rpm, 00:00:30 to wash the spin column membrane. Discard the flow-through. 30s

15 Add 500 µL Buffer RPE to the RNeasy spin column. Gently rotate the tube while applying the RPE buffer to the inside of the column walls, rather than directly to the filter, to ensure removal of any residues. Close the lid, and centrifuge for 10000 rpm, 00:00:30 . Discard the flow-through. 30s

16 Add 500 µL Buffer RPE to the RNeasy spin column. Gently rotate the tube while applying the RPE buffer to the inside of the column walls, rather than directly to the filter, to ensure removal of any residues. Close the lid, and centrifuge for 10000 rpm, 00:02:00 . Discard the collection tubes. 2m

17 Place the RNeasy spin column in a new 2 ml collection tube. Centrifuge at full speed for , 00:05:00 to dry the membrane. 5m

18 Place the RNeasy spin column in a new collection tube (supplied). Add 50 µL RNase-free water directly to the center of spin column membrane. Incubate for 00:05:00 at Room temperature . Then centrifuge for 10000 rpm, 00:01:00 to elute the RNA. 6m

19 Repeat step 18 with another 50 uL.

20 Quantify and evaluate the quality of the RNA using a NanoDrop spectrophotometer and a Bioanalyzer.

21 The RNA can now be stored at -80°C for use in downstream sequencing applications.