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RNA extraction from diatom P. multistriata

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ABSTRACT

RNA extraction protocol from diatom P. multistriata

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1 Collect 20/40 million cells

2 Harvest cells by filtration onto 1.2 µm pore size filter

3 Cut the filter into two halves and store each half in separate 2 mL eppendorf tube.

4 Add 1 mL of TRIzol® Reagent (for 5-10 × 10^6 cells) to eppendorf with half filter and vortex briefly.

TRIzol Reagent **Thermo Fisher Scientific Catalog #15596026**

5 Flash freeze the eppendorf immediately in liquid nitrogen.
The eppendorf can be stored at -80 °C for later extraction.

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Note: Do not wash cells before addition of TRIzol® Reagent.

Defrost the samples at room temperature (use thermoshaker for 15 sec at 60 °C if defrosting takes longer).

DO NOT PROCESS MORE THAN 6 SAMPLES AT SAME TIME.

6 Add 0,2g glass beads and put on thermoshaker at 60 °C for 10 min with maximum rpm (1400).
⊠Glass beads, acid-washed, 425-600 µm (30-40 U.S. sieve) **Sigma Aldrich Catalog #G8772-100G**

7 Centrifuge briefly for one minute to get rid of beads and filter and move the supernatant into 2 mL eppendorf tube.

8 Add 200 ul of CHLOROFORM per 1 ml of Trizol

9 Shake the eppendorf vigorously for 15 seconds

10 Incubate for 15 minutes at room temperature.

11 Centrifuge the eppendorf for 15 minutes at 10600 rpm (12,000 g) **AT MAXIMUM** , at 4 °C.

12 Transfer 500 ul (half the volume of Trizol used originally) of uppermost aqueous layer (contains RNA) into new 2mL eppendorf.

Do not disturb middle colorless phase and lowermost pink phase while pipetting (these phases contain proteins and DNA).

13 Add equal volume of ISOPROPANOL (500 ul) and invert the tube to mix.

Don't shake.

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14 Incubate at room temperature for 10 minutes

(samples can be stored at 4 °C overnight).

15 Centrifuge for 10 minutes at 10600 rpm at 4 °C.

16 Remove supernatant leaving RNA pellet undisturbed.

17 Add 1 mL of 75% ethanol. Invert the tube gently to wash the pellet

(samples can be stored at -20 °C for long time).

18 Centrifuge for 10 minutes at 8500 rpm (7500 g) at 4 °C.

19 Remove supernatant leaving RNA pellet undisturbed.

20 Allow the pellet to dry for 20-30 minutes to remove all the traces of ethanol.

Care should be taken not to over dry the pellet.

21 Re-suspend the pellet in 20-30 ul of RNA-se free water and dissolve the pellet by pipetting up and down.

For better re-suspending, incubate the samples for 5-10 minutes at 55-60 °C.

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