RNA extraction from the pennate diatom Asterionella formosa

Carine Puppo,Tomas Voisin,Brigitte Gontero

Abstract

Asterionella formosa is a freshwater diatom and as such as a silica cell wall. Therefore classical protocols for RNA extraction do no work. We have settled this protocol that gives high quality RNA (tested by NGS). This protocol was also applied to marine diatoms, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* and gave very good yields.

Citation: Carine Puppo,Tomas Voisin,Brigitte Gontero RNA extraction from the pennate diatom Asterionella formosa. **protocols.io**
dx.doi.org/10.17504/protocols.io.nu8dezw
Published: 23 Mar 2018

Materials

TRIzol Reagent 15596026 by Thermo Fisher Scientific

SV Total RNA Isolation System (50 preps) Z3100 by Promega

Protocol

Before starting

Step 1.

This protocol requires 90% ethanol kept at -20°C. All the equipment (pipetman, bench....) has to be decontaminated with a RNAse AWAY solution. The tips and tubes have to be RNAse free. Wear gloves during all the procedure.

Preparing cells

Step 2.

1L of *Asterionella formosa* culture should be grown to an optical density at 600nm (OD_{600}) of 0.12.

Step 3.

Harvest the cells by centrifugation at 3 200g at 16°c in a swinging rotor for 15min. Discard the supernatant. With the pellet, follow the protocol below.

RNA extraction

Step 4.

Add 4 ml of TRIzol Reagent to the harvested cells (roughly 360 mg).

REAGENTS
TRIzol Reagent 15596026 by Thermo Fisher Scientific

Step 5.

Incubate at room temperature for 5 min.

Step 6.

Split the 4 mL samples into 4 samples of 1 mL each (in eppendorf tubes).

Step 7.

Add 200 µL of chloroform to each sample and shake vigorously.

NOTES
Brigitte Gontero 16 Mar 2018

For this step, pure chloroform is necessary. A solution of chloroform and isoamyl alcohol should not be used as a substitute.

Step 8.

Incubate at room temperature for 10 min.

Step 9.

Centrifuge for 15 min at 4°C at maximal speed in a benchtop microcentrifuge.

Step 10.

Collect the aqueous phase from each tube whith a needle syringe (top phase, about 0.6 mL per tube) and split into aliquots of 200 µL each.

Step 11.

Add 375 µL of RNA dilution buffer from the SV Total RNA Isolation System kit (blue solution) to each aliquot and gently mix by shaking.

REAGENTS
SV Total RNA Isolation System (50 preps) Z3100 by Promega

Step 12.

Centrifuge for 10 min at maximal speed in a benchtop microcentrifuge at 4°C.

Step 13.

Collect the supernatant, which should be about 500 µl per tube. A blue pellet may sometimes be present.

Step 14.

Add 250 µL of 90% ethanol kept at -20°C to each supernatant sample (of 500 µL) from the previous step.

Step 15.

Continue from step 7 of the SV Total RNA Isolation System Protocol.

Tip as many tubes as possible into the Spin Basket Assembly (between 12 and 13 tubes).

7. Transfer mixture to Spin Basket Assembly and centrifuge for 1 minute. Discard eluate.
8. Add **600µl** of **RNA Wash Solution (RWA)** (+ ethanol). Centrifuge for 1 minute and discard the eluate.
9. Prepare **DNase incubation mix** using the table below:

Solution	Volume	×	Number of Preps	=	Total
Yellow Core Buffer	40µl				
$MnCl_2$, 0.09M	5µl				
DNase I	5µl				

*Mix gently (pipet); do **not** vortex.*

10. Apply **50µl** of DNase mix to membrane. Incubate at RT for 15 minutes.
11. Add **200µl DNase Stop Solution (DSA)** (+ ethanol) and centrifuge for 1 minute.
12. Add **600µl RNA Wash Solution (RWA)**; centrifuge for 1 minute. Empty.
13. Add **250µl RNA Wash Solution (RWA)**; centrifuge for 2 minutes. Transfer Spin Basket to Elution Tube.
14. Add **100µl Nuclease-Free Water** to membrane. Centrifuge for 1 minute to elute the RNA and store at –70°C.

RT: room temperature
Centrifugation: 12,000–14,000 × *g* (at RT)

REAGENTS

SV Total RNA Isolation System (50 preps) Z3100 by Promega