Modified total RNA extraction for Heterosigma akashiwo using the Qiagen RNeasy kit

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Abstract

This protocol is a modified version of the Qiagen RNeasy assay for isolating total RNA from Heterosigma akashiwo. We used this protocol to obtain high quality RNA for downstream analysis such as quantitative PCR.

Citation: Monica Accerbi,Vinay K Nagarajan,Kathryn Coyne,Pamela J Green Modified total RNA extraction for Heterosigma akashiwo using the Qiagen RNeasy kit. **protocols.io**

dx.doi.org/10.17504/protocols.io.hipb4dn

Published: 30 Sep 2017

Protocol

Step 1.

Starting material: 1 ml culture with about 400,000 cells

Step 2.

To the cells add 1 ml of Buffer RLT with 10 µl β-mercaptoethanol

Step 3.

Centrifuge through a QIAshredder column (750 µl at a time) at 11,000 rpm for 1’ and collect FT (flow through) in a 5 ml tube

Step 4.

To the collected FT add 2 ml of 70% (v/v) ethanol and mix at room temperature

Step 5.

Centrifuge through a RNeasy spin column (750 µl at a time) at 11,000 rpm for 1’ and discard FT

Step 6.

Add 750 µl Buffer RW1 and centrifuge at 11,000 rpm for 1’ and discard FT

Step 7.

Wash column with 500 µl Buffer RPE, centrifuge at 11,000 rpm for 1’ and discard FT

Step 8.

Repeat step 7

Step 9.

Centrifuge once more at 11,000 rpm for 1’ to dry the membrane and remove residual ethanol

Step 10.

Elute total RNA with 15 µl DEPC (Diethyl pyrocarbonate) -treated H_2O

Expected yield

Step 11.

Total RNA yield using this method is around 350 ng with 260:280 nm $\geq$ 1.8

For larger volumes of starting material

Step 12.

Centrifuge the starting material at 2,000 to 3,000 rpm for 2’ at room temperature

Discard supernatant and resuspend pellet in the residual liquid, about 1 ml

Proceed as above, steps 1 to 9. Elute total RNA with 25-30 µl DEPC-treated H2O