



## RNA extraction from adult Aiptasia [↗](#)

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Aiptasia-Symbiodinium Model System

Kelsey Speer

### ABSTRACT

A protocol for extracting RNA from Aiptasia, modified from the Weis lab protocol by Angela Poole and Mauricio Rodriguez-Lanetty. Note that the final product has significant DNA contamination and needs to be cleaned up via DNase treatment.Â

### TAGS

RNA extraction

Aiptasia

### EXTERNAL LINK

[http://people.oregonstate.edu/~weisv/assets/trizol\\_rneasyhybrid.pdf](http://people.oregonstate.edu/~weisv/assets/trizol_rneasyhybrid.pdf)

### THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Rodriguez-Lanetty, M., Phillips, W.S., Weis, V.M., 2006. Transcriptome analysis of a cnidarian-dinoflagellate mutualism reveals complex modulation of host gene expression. BMC Genomics 7, 23. doi:10.1186/1471-2164-7-23

### PROTOCOL STATUS

#### Working

We use this protocol in our group and it is working

### MATERIALS

NAME	CATALOG #	VENDOR
RNeasy Mini Kit	74104	Qiagen
TRIzol Reagent	15596026	Thermo Fisher Scientific
Micropestle	<a href="#">View</a>	

### SAFETY WARNINGS

All Trizol steps should be performed in the hood. All Trizol waste should be treated as hazardous.

### BEFORE STARTING

Make sure to setup a clean area in the fume hood, wipe down all pipettes with RNase Away/Zap, and cool down the centrifuge.

- 1 Homogenize starting material in Trizol using a micropestle. Trizol volume should be ~ 1 mL per 0.1 g tissue. Once the homogenate is uniform, incubate at room temperature for 5 minutes.

#### ▲ SAFETY INFORMATION

All Trizol steps (1-5) should be performed in a fume hood and all Trizol waste should be treated as hazardous.

- 2 Centrifuge samples for 10 minutes at 12,000 x g at 4°C. This step will pellet any debris and polysaccharides. Transfer the supernatant to a new tube and discard the pellet.
- 3 Add chloroform to the sample (0.2 mL per 1 mL of Trizol used) and shake vigorously for 20 seconds. Incubate samples at room temperature for 2-3 minutes.
- 4 Centrifuge samples for 18 minutes at 10,000 x g at 4°C.

**NOTE**

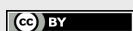
\*Note: past this step you should be careful of RNase contamination\*

- 5 Carefully remove the top aqueous (clear) layer and transfer to a new sterile, RNase-free tube.

**NOTE**

IMPORTANT: When you take your samples out of the centrifuge, you will see three layers: aqueous (clear, top), interphase (white, middle) and organic (red, bottom). Stay away from the white middle layer - that is where the DNases and RNases are. It is better to leave a little of the aqueous layer behind than to risk contamination.

- 6 **Slowly** add an equal volume of 100% RNase-free EtOH and mix gently by pipetting or inverting tube.
  - 7 Load sample (up to 700 µL) into an RNeasy spin column (Qiagen) and spin at room temperature for 1 min at 8,000 x g. Discard flow through.
- NOTE**
- If your total volume exceeds 700 µL, repeat this step until all of the sample has passed over the column.
- 8 Add 700 µL of Buffer RW1 to the column and spin at room temperature for 1 min at 8,000 x g. Discard flow through.
  - 9 Transfer spin column to a new collection tube and add 500 µL of Buffer RPE (check that ethanol has been added) and spin at room temperature for 1 min at 8,000 x g. Discard flow through.
  - 10 Add 500 µL of Buffer RPE and spin at room temperature for 2 min at 8,000 x g. Discard flow through.
  - 11 Spin for an additional 1 minute at 8,000 x g to get rid of remaining buffer in column.
  - 12 Transfer the spin column to a new, RNase-free 1.5 mL collection tube and pipette 30-50 µL of RNase-free water over the center of the membrane (do not touch the membrane with your pipette tip!). Let the sample sit at room temperature for 1 minute and then spin for 1 minute at 8,000 x g to elute RNA.
  - 13 Discard the spin column and store your RNA at -80°C until further use.



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