# RNA extraction from adult Aiptasia

Kelsey Speer[1], Angela Poole[2], modified from Weis lab protocol by Mauricio Rodriguez-Lanetty[3]
[1]University of Pennsylvania, [2]Berry College, [3]Oregon State University

dx.doi.org/10.17504/protocols.io.sp5edq6

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

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 | View | |

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 0.1g 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 4Â°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 4Â°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 Î¼L) 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 Î¼L, repeat this step until all of the sample has passed over the column.

8 Add 700 Î¼l 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 Î¼l 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 Î¼l 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 Î¼l 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 -80Â°C until further use.