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RNA Extraction with Trizol

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Dakota Betz[1]

[1]ucsd

Rouse Lab

Marina McCowin
UCSD- Scripps Institution of Oceanography

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Protocol status: Working
We use this protocol and it's working

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Abstract

This is a protocol for RNA extractions (using Trizol). A maximum of 3 samples can be extracted at once using this protocol. The entire protocol should be run in the fumehood. This protocol is time- and temperature-sensitive, so read all instructions before attempting and make sure you are adequately prepared.

Guidelines

A maximum of 3 samples can be extracted at once using this protocol.

Materials

- RNase Away
- RNA free tubes (1.6 and 2 ul)
- Petri dishes
- Trizol
- 100% ethanol (new every time)
- RNase free water (new every time)
- Direct-zol RNA miniprep kit (Zymo research) ← contains all other reagents needed

Troubleshooting

Safety warnings

Trizol is BAD--conduct entire extraction protocol in the fume hood! You will need to move the centrifuge, bead basher, and other equipment into the hood before starting. Make sure you have icepacks available as this protocol is time- and temperature-sensitive.

Before start

Celean all surfaces, tools, and gloves with RNase Away. Don't forget to clean pipettes, plastic pipettes, ice blocks, centrifuge, etc.
Make sure buffers have been prepped if it's a new kit (instructions in kit for which reagents/how much to add to each).

Homogenize Tissues

1 Add **770 uL** of **Trizol** to bead bashing tube (1 per sample).

2 Pour tissue into a clean Petri dish, keep on cooling block (flat ice pack).

3 Transfer pieces of tissue to bead bashing tube, blot RNAlater with a kim tissue.
May be a very small amount of tissue (if so use all) and blot until it's REALLY dry. Do all of this on top of an ice pack to keep it cold.

4 Beadbash **3-4 times for 15 sec** at max speed. Cool down **5-10 seconds** in between, placing tube in/on ice block. Stop as soon as tissue is dissolved!

5 Transfer liquid to a 1.6 mL RNase free tube and centrifuge 12000 x g, 00:02:00 .
Use block from lab freezer to keep tubes cold.

6 Transfer supernatant to a new 1.6 mL RNase free tube.

RNA purification & DNase I treatment

7 Add **700 uL** of **100% ethanol** to each tube. Vortex.

8 Load **700 uL** into ZymoSpin IIC Column in the 2mL-size RNase-free collection tube. Use this size for all remaining steps. Centrifuge 12000 x g, 00:01:00 .

> Note
>
> For sea cucumbers, urchins, or other tissues that might clog the filter, split them into two ZymoSpin IIC Columns instead of doing step 9: i.e., treat like two "separate" samples for the rest of the protocol, then combine them at the end.

9 1) Discard flow-through. Repeat with remaining **700 uL** (if applicable). If first half did not flow through completely, centrifuge 12000 x g, 00:02:00 .

10 2) Transfer column to RNase-free tube and do in-column digestion. Wash with **400 uL** of **RNA Wash Buffer**. Centrifuge 12000 x g, 00:00:30 , discard flow-through.

11 3) Add **80 uL** of **DNase I Reaction Mix** (75 µL DNA digestion Buffer (kit), 5 µL DNase I (freezer, green cap); scaled) directly to the column. Incubate for 00:15:00 at room temperature . Centrifuge 12000 x g, 00:00:30 .

12 4) Add **400 uL** of **Direct-zol RNA PreWash**. Centrifuge 12000 x g, 00:01:00 .

13 5) Discard flow through. Repeat previous step. (Pre-wash and centrifuge again).

14 6) Add **700 uL** of **RNA Wash Buffer**. Centrifuge 12000 x g, 00:01:00 . Discard flow-through. Centrifuge again in emptied collection tube 12000 x g, 00:02:00 .

15 7) Transfer column to labeled RNase free tube.

16 8) Add **26 uL** of **RNase-free water** to column. Incubate 00:03:00 at room temperature . Centrifuge 12000 x g, 00:01:00 .

17 9) Combine tubes if applicable (see note on step 8). Take **5 uL** for QC/Qubit. The kit is called “Qubit RNA HS” and has weirdly clear dye and different standards.

18 10) Store everything at -80 °C