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Coral tissue and skeleton Trizol RNA extraction

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1 Works for me dx.doi.org/10.17504/protocols.io.bi9ikh4e

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ABSTRACT

This protocol was modified from the TRIzol protocol provided by Invitrogen.

https://assets.thermofisher.com/TFS-Assets/LSG/manuals/trizol_reagent.pdf

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MATERIALS TEXT

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Ethanol (100%, Molecular Biology Grade) **Fisher Scientific Catalog #BP2818500**

nuclease free water **Contributed by users**

RNA Clean & Concentrator-5 Kit **Zymo Research Catalog #R1015**

5M NaCl **Ambion Catalog #AM9760G**

Sodium Citrate **Sigma Catalog #71402**

RNaseZap™ RNase Decontamination Solution **Thermo Fisher Scientific Catalog #AM9780**

TRIzol™ Reagent **Thermo Fisher Catalog #15596026**

Phasemaker™ Tubes **Thermo Fisher Catalog #A33248**

GlycoBlue™ Coprecipitant (15 mg/mL) **Thermo Fisher Catalog #AM9516**

Isopropanol Molecular Biology Grade **Contributed by users**

Chloroform Molecular biology grade **Contributed by users**

Refrigerated centrifuge for 2mL tubes **Contributed by users**

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This protocol was modified from the TRIzol protocol provided by Invitrogen.

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Solutions to prepare ahead of time

1 Make high-salt buffer (0.8M NaCitrate/1.2 M NaCl).

Add **23.5 g NaCitrate** to **24 mL** of **5 Molarity (M) NaCl** .

Add water to a final volume of **100 mL** .

Autoclave and aliquot into working solutions. Each sample will need **250 µl high salt buffer** .

Set Up

2 ⚠

Cleaning and tips to avoid contamination:

Clean UV hood and fume hood with bleach, ethanol, RNaseZap, and sterilize UV hood for 15 minutes.

Clean all pipette tips with bleach, ethanol, RNaseZap.

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Keep UV hood and fume hood pipettes separate.

Use reagents that are dedicated for RNA work.

Prepare solutions and aliquots in clean, sterilized UV hood.

Use only nuclease free plasticware.

Use filter pipette tips designated exclusively for RNA work.

3 

Warning:

Trizol reagent contains guanidinium thiocyanate and acid phenol. All procedures involving the transfer of Trizol or chloroform should be performed in a chemical fume hood. Waste should be treated as hazardous and disposed of properly.

4 Using molecular biology grade ethanol, prepare fresh **75 % (v/v) ethanol** (2mL per sample). Place 75% ethanol in **-20 °C** freezer so that it will be cold when used in Step 18.

5 Cool down centrifuge to **4 °C** .

RNA/DNA Separation

6 Thaw samples.

Since coral tissue was previously homogenized with the tissue tearor during separation, it should not be too viscous when pipetted. It might be thick, but you should be able to pipette it with ease. Homogenize again with tissue tearor if it is too viscous. When the tissue is not well homogenized, it will be difficult to separate the aqueous phase in Step 10.

7 **In fume hood,** add **1 mL Trizol** to sample aliquots prepared during tissue/skeleton separation (approximately 200-500µl of tissue solution and 200-500cm^3 of skeleton).

If aliquots have more sample than desired for extraction, transfer sample into a new nuclease free 2mL tube and add 1mL Trizol.

If desired, Phasemaker tubes can be used here instead of 2mL microcentrifuge tubes to facilitate phase separation and help prevent DNA contamination.

8 For skeletal samples, add **1 µl** to **5 µl** of 6M HCl (18.5% HCl) to sample/Trizol mixture.

9 Vortex sample/Trizol mixture for **00:00:30** . It is important thoroughly homogenize the sample in Trizol. Incubate samples for **00:05:00** to allow for complete dissociation of the nucleoproteins complex.

10 **In fume hood**, add **200 µl chloroform** per 1mL of Trizol reagent. Shake samples vigorously by hand and then

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vortex briefly. The solution should be well mixed and milky.

Incubate for **00:03:00** at room temperature.

11 Centrifuge **12000 x g, 4°C, 00:15:00** . The mixture will separate into a lower red phenol-chloroform, an interphase, and a colorless upper aqueous phase.

12 **In fume hood**, carefully transfer the upper aqueous phase containing the RNA to a new 2mL DNase/RNase free tube. Avoid transferring any of the interphase or organic layer into the pipette when removing the aqueous phase.

RNA Isolation

13 **In fume hood,** add **250 µl isopropanol** and **250 µl high salt solution (0.8 NaCitrate/1.2M NaCl)** to the aqueous phase, per 1mL of Trizol used in lysis.

Adding the high salt solution allows polysaccharides and proteoglycans to remain in soluble form (Chomczynski & Mackey, 1995), preventing them from co-precipitating with the pellet.

14 Add **1 µl glycoblue (20mg/ul)** . Vortex briefly.

15 Incubate at room temperature for **00:10:00** .

16 Centrifuge at **12000 x g, 4°C, 00:10:00** . The RNA precipitate forms a blue gel-like pellet on the side or bottom of the tube. If glycoblue was not used, the pellet will be clear/white.

17 Without disturbing the pellet, remove and discard the supernatant. The pellet might be barely visible so use caution when pipetting. It is helpful to know the side of the tube your pellet will be on, based on its position in the centrifuge.

(After this step, move to the UV sterilized hood, instead of the fume hood).

18 Using the ethanol prepared in Step 3, resuspend the pellet in **1 mL cold 75% ethanol** per 1mL of Trizol. Vortex briefly.

19 Centrifuge at **7500 x g, 4°C, 00:05:00** .

20 Without disturbing the pellet, remove and discard the supernatant.

21 Repeat the ethanol wash by resuspend the pellet in **1 mL cold 75% ethanol** per 1mL of Trizol. Vortex briefly.

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22 Centrifuge at **7500 x g, 4°C, 00:05:00** .

23 Without disturbing the pellet, remove and discard the supernatant.

24 Air dry pellet for **00:05:00** to **00:10:00** by leaving the lid open.

25 Solubilize RNA by resuspending the pellet in **50 µl nuclease free water** by pipetting up and down. Incubate on heat block at **55 °C** for **00:10:00**

Note: For some coral species, a white insoluble pellet may form during precipiation. Using a clean and concentrator kit (e.g. Zymo) can help to remove insoluable co-precipitants and improve RNA solubilization. See optional section below.

26 Check RNA yields.

RNA concentration should range from 50-300 ng/µl. If yield is low (<50 ng/µl) use clean and concentrate protocol below.

27 Store at **-80 °C** .

RNA Clean & Concentrate

28 For 50µl of sample, add **100 µl RNA Binding Buffer** and vortex briefly.

29 Add **150 µl 100% Ethanol** and vortex briefly.

30 Transfer solution to spin column and collection tube.

31 Centrifuge at **10000 x g, Room temperature , 00:00:30** and discard flow-through.

32 Add **400 µl RNA Prep Buffer** to column and centrifuge at **10000 x g, Room temperature , 00:00:30** . Discard the flow-through

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33 Add **700 µl RNA Wash Buffer** to column and centrifuge at **10000 x g, Room temperature , 00:00:30** .
Discard the flow-through

34 Centrifuge at **10000 x g, Room temperature , 00:01:00** to fully remove wash buffer.

35 Transfer the column into a new nuclease-free tube.

36 Add desired volume of nuclease free water to column and incubate at room temperature for 5 minutes.

Note: For reverse transcription, aim to have ≥ 500ng of RNA for the RT reaction and ≥ 500ng of RNA for the noRT control. For solutions with low yield, eluding in **35 µl nuclease free water** it recommended, so that each RT/noRT reaction can contain 12µl of sample, leaving 11µl that can be used for quantification and to check for DNA contamination.

37 Centrifuge at **10000 x g, Room temperature , 00:00:30** .

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