Total RNA Isolation from oyster tissue using RNAzol RT.

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Abstract

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Materials

- DEPC-Treated Water 4387937 by Contributed by users
- Isopropanol by Contributed by users
- 75% Ethanol by Contributed by users
- P1000 micropipets and 1 ml filter tips by Contributed by users
- P200 micropipets and 200 µl filter tips by Contributed by users
- Gloves by Contributed by users

  RNAzol RT RN 190 by Molecular Research Center, Inc.
- Protective eyewear by Contributed by users

Protocol

Step 1.
1. Read the SOPs for each of the reagents used in this protocol.

Step 2.
2. Read the manufacturer's [RNAzol RT protocol for Total RNA Isolation](https://github.com/sr320/LabDocs/blob/master/protocols/Commercial_Protocols/MRC_RNAzol-RT-May-2014.pdf).

Step 3.
3. Read *this* protocol.

Step 4.
4. Verify sufficient quantities/availability of reagents/equipment.

Step 5.
5. Wear clean gloves and change gloves frequently.

Step 6.
6. Aliquot 500uL of RNAzol RT to pestle tubes and store on ice.

AMOUNT
500 µl Additional info:

REAGENTS
RNAzol RT RN 190 by Molecular Research Center, Inc.

Step 7.
7. Transfer tissue to pestle tubes containing RNAzol RT.

Step 8.
8. Homogenize immediately with disposable pestle.

Step 9.
9. Immediately add additional 500uL of RNAzol RT to pestle tube.

AMOUNT
500 µl Additional info:

REAGENTS
RNAzol RT RN 190 by Molecular Research Center, Inc.

Step 10.
10. Vortex 15s.

Step 11.
11. Add 400uL of 0.1% DEPC-treated H2O.

AMOUNT
400 µl Additional info:

REAGENTS
DEPC-Treated Water 4387937 by Contributed by users

Step 12.
12. Vortex 15s.

Step 13.
13. Incubate at room temperature (RT) for 15mins.

Step 14.
14. Centrifuge 12,000g for 15mins @ RT.

Step 15.
15. Transfer 750uL of supernatant (do not disturb pellet) to sterile 1.7mL snap-cap tube. (Discard remaining liquid in RNAzol RT Hazardous Waste container in fume hood. Leave old tube open in fume hood over night and then discard in regular trash.)

AMOUNT
750 µl Additional info:

Step 16.
16. Add 1 volume of isopropanol.

AMOUNT
750 µl Additional info:

Step 17.
Read the SOPs for each of the reagents used in this protocol.

Step 18.
Read the manufacturer's [RNAzol RT protocol for Total RNA Isolation](https://github.com/sr320/LabDocs/blob/master/protocols/Commercial_Protocols/MRC_RNAzol-RT-May-2014.pdf).

Step 19.
Read *this* protocol.

Step 20.
Verify sufficient quantities/availability of reagents/equipment.

Step 21.
Wear clean gloves and change gloves frequently.

Step 22.
Aliquot 500uL of RNAzol RT to pestle tubes and store on ice.

Step 23.
Transfer tissue to pestle tubes containing RNAzol RT.

Step 24.
Homogenize immediately with disposable pestle.

Step 25.

Immediately add additional 500uL of RNAzol RT to pestle tube.

Step 26.
Add 400uL of 0.1% DEPC-treated H2O.

Step 27.
Vortex 15s.

Step 28.
Incubate at room temperature (RT) for 15mins.

Step 29.
Centrifuge 12,000g for 15mins @ RT.

Step 30.
Transfer 750uL of supernatant (do not disturb pellet) to sterile 1.7mL snap-cap tube. (Discard remaining liquid in RNAzol RT Hazardous Waste container in fume hood. Leave old tube open in fume hood over night and then discard in regular trash.)

Step 31.
Add 1 volume of isopropanol.

Step 32.
Vortex 5s.

Step 33.
Incubate @ RT for 15mins.

Step 34.
Centrifuge 12,000g for 10mins @ RT.20. Discard supernatant; do not disturb pellet.

Step 35.
Add 400uL of 75% ethanol, centrifuge 4,000g for 3mins @ RT.