Ocean Ridge Biosciences

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RNA Isolation for RNA Sequencing and Microarray Applications

Please read the manual accompanying the Trizol Reagent carefully. These protocols are adapted from that manual.

Part A. Homogenization of Tissue Stored in RNALater Using Trizol

1. Remove frozen tissue (in RNAlater) from freezer and thaw at room temperature.
2. After tissue is thawed, move to ice.
3. Transfer tissue to weigh boat, remove excess RNALater and weigh.
4. Transfer tissue (20-100 mg) to 12 x 75 mm polypropylene tube stored on ice.
5. Add 1.0 ml of Trizol (Invitrogen, part# 15596-026, 100 ml) to the tube containing tissue.
6. Immediately homogenize with Omni TH on medium setting for 15-30 seconds.
7. Examine tube to insure all tissue has been disrupted
8. Transfer homogenate to a microfuge tube at room temperature.

Part B. Trizol RNA Isolation

Note, if RNA recovery is expected to be less than 5 ug, then add 20 ug of Molecular Biology Grade Glycogen prior to further processing.

1. Add 0.2 ml chloroform and vortex for 15 sec.
2. Incubate mixtures at 15 to 30C for 2-3 min.
3. Spin at full speed (12,000 g) in microfuge at 4C (in refrigerator) for 15 min.
4. Remove 450-500ul of aqueous phase and transfer to a new tube.
   Note, do not touch or collect material from interphase.
5. Add an equal volume (450-500 ul) of isopropyl alcohol to the tube and vortex for 5 seconds.
6. Incubate tubes at 15 to 30C for 2-3 min.
   Note: Incubate tubes overnight at -20C here if RNA will be used for miRNA analysis.
7. Spin at 12,000 g for 10min at 4C.
8. Carefully pour off supernatant, while observing that pellet is not lost from bottom of tube.
   Note, supernatant can be poured in to labeled microfuge tube, to prevent accidental loss of pellet.
9. Add 500ul of 75% ethanol.
10. Vortex to partially re-suspend pellet.
11. Spin for 2-3 min at RT.
12. Pour off ethanol wash while carefully observing that pellet is not lost.
    Note, ethanol wash can be poured in to labeled microfuge tube, to prevent accidental loss of pellet.
13. Spin briefly again to collect residual ethanol at the bottom of tube.
14. Remove remaining ethanol with P200 pipette, carefully avoiding pellet.
15. Lay tubes down on tissue paper with tops open. Let air dry for 5 min.
16. Re-suspend in minimum of 1 ul Qiagen RNase-free water per 1-5 ug of RNA expected.
17. Measure OD at 260 nm and 280 nm of a 1:50 dilution of the RNA sample.
    Use 10 mM Tris pH 7.5 as blank and as the diluent, for the OD reading.
18. Calculate concentration in ng/ul by multiplying by 40 ug/ml = 1 OD and by the dilution.

For small RNA applications stop here; the remainder of processing will occur at ORB.

Shipping

Select a box for shipment that has an external dimension of at least 25 cm in each dimension. The box should have a sturdy cardboard exterior and inner Styrofoam box with wall thickness of at least 2.5 cm. Fill the storage box with 1.5 kg of dry ice. Cool a 133 mm x 133 mm x 48 mm vial storage box in the shipping container. Transfer the frozen sample tubes to the vial storage box. Fill in the free areas of the vial storage box with dry ice and secure the vial storage box top over the samples using wire or string. Fill the Styrofoam shipping container with additional dry ice, and packing peanuts (if necessary) in order to minimize free space in the package. The total dry ice content should be at least 2.5 kg. Use FedEx overnight delivery to ship the package to ORB at the address below. Affix the dry ice sticker to the exterior of the package and record the same dry ice weight on the sticker as was used during set-up of the shipment (may require metric to English unit conversion). *Shipping address can be located on Page 2.*

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