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Preparing RNA from Broncho Alveolar Lavage Fluid (BALF) samples for small RNA Profiling

1. Pellet the cells by centrifuging the BALF samples at 400 g for 10 min at 4C.
   Reference: Lewis, S., Singh, D., and Evans, C.E., (2009) "Cyclic hydrostatic pressure & cotton particles stimulate synthesis by human lung macrophage of cytokines in vitro", Respiratory Research, 10:44
2. Discard the supernatant.
   Note: Pelleted cells can be processed using a Trizol method or using the miRvana isolation kit (Ambion, Part #: AM1560).
   (https://tools.thermofisher.com/content/sfs/manuals/cms_055423.pdf)

RNA Isolation - Trizol Method:

3. Homogenize the cells, in 1 ml of Trizol (Invitrogen, Part #: 15596-026) using Omni TH or other rotar-stator homogenizer.
   Note: Please Read the Manual Accompanying the Trizol Reagent Carefully. These protocols are adapted from that manual.
4. Add 0.2 ml chloroform and vortex for 15 sec.
5. Incubate mixtures at 15 to 30C for 2-3 min.
6. Spin at full speed (12,000 g) in microfuge at 4C (in refrigerator) for 15min.
7. Remove 450-500ul of aqueous phase and transfer to a new tube.
   Note: Do not touch or collect material from interphase
8. Add 1ul of 20ug/ul Glycogen (Invitrogen, Part #: 10814-010) per sample and mix well by vortexing.
   Note: Glycogen co-precipitates with nucleic acids and increases the visibility of the nucleic acid pellet.
9. Add an equal volume (450-500 ul) of isopropyl alcohol to the tube and vortex for 5 seconds.
10. Incubate them at 15 to 30C for 2-3 min.
    Note: Incubate tubes overnight at -20C here if RNA will be used for miRNA analysis.
11. Spin at 12,000 g for 10min at 4C.
12. Carefully remove liquid.
13. Add 500ul of 75% ethanol & vortex to partially re-suspend pellet.
14. Spin for 2-3 min at RT.
15. Pour off ethanol wash while carefully observing that pellet is not lost.
16. Spin briefly again to collect residual ethanol at the bottom of tube.
17. Remove remaining ethanol with P200 pipette, carefully avoiding pellet.
18. Lay tubes down on tissue paper with tops open. Let air dry for 5 min.
19. Re-suspend in 30 ul of Qiagen RNase-free water.
20. To prepare samples for shipping, transfer samples to RNA stable tubes (Biomatrica, Part #: 93221-001) and dry them down according to manufacturers instructions. (See link below)
    http://www.biomatrica.com/media/rnastable/protocols.pdf

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 found on Page 2.

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