

RNA Extraction

Step 1. Add 600 µL Lysis/Binding Buffer to the cell or tissue lysate. Then add 30 µL miRNA Homogenate Additive to homogenate, and mix well by vortexing or inverting the tube several times. Leave the mixture on ice for 10 min.

Step 2. Add a volume of Acid-Phenol: Chloroform that is equal to the lysate volume before addition of the miRNA Homogenate Additive. Centrifuge for 5 min at maximum speed (10,000 x g) to separate the aqueous and organic phases. Carefully remove the aqueous (upper) phase without disturbing the lower phase, and transfer it to a fresh Tube.

Step 3. Add 1.25 volumes of room temperature 100% ethanol to the aqueous phase.

Step 4. Pipet the lysate/ethanol mixture (from the previous step) onto the Filter Cartridge (Note: Up to 700 µL can be applied to a Filter Cartridge at a time.). Centrifuge for 30 sec at 13,000 rpm to pass the mixture through the filter. Discard the flow-through.

Step 5. Apply 350 µL miRNA Wash Solution 1 to the Filter Cartridge and centrifuge for 30 sec at 13,000 rpm. Discard the flow-through from the Collection Tube, and replace the Filter Cartridge into the same Collection Tube.

Step 6. The 10 µL DNase I and 70 µL Buffer RDD QIAGEN (#79254) were mixed. Then the mixture was added to the Filter Cartridge. Leave it at the room temperature for 15 min.

Step 7. Apply 350 µL miRNA Wash Solution 1 to the Filter Cartridge and centrifuge for 30 sec at 13,000 rpm. Discard the flow-through from the Collection Tube, and replace

the Filter Cartridge into the same Collection Tube.

Step 8. Apply 500 µL Wash Solution 2/3 and centrifuge for 30 sec at 13,000 rpm. Draw it through the Filter Cartridge as in the previous step. Repeat with a second 500 µL aliquot of Wash Solution 2/3.

Step 9. Spin the assembly for 1 min to remove residual fluid from the filter. Transfer the Filter Cartridge into a fresh Collection Tube. Apply 100 µL of pre-heated (95°C) Elution Solution to the center of the filter. Leave it at the room temperature for 2 min. Spin for ~20–30 sec at maximum speed to recover the RNA. Collect the eluate (which contains the RNA) and store it at –70°C.