RNA Isolation

Qiu Lab

Updated 6/2016

RNA Isolation

BEFORE STARTING

1. The first time you use the kit, you will need to make several solutions. All should be prepared to RNAse/DNAse free water
    a. 70% EtOH
        i. 35 mL Absolute (200 proof) ethanol
        ii. 15 mL water
    b. 80% EtOH
        i. 40 mL Absolute (200 proof) ethanol
        ii. 10 mL water
2. You will need to add 4 volumes EtOH (200 proof) to buffer RPE.
3. DNAse I needs to be prepared and aliquoted.
    a. Add 550 µL of RNAse/DNAse free water to the lyophilized enzyme.
    b. Mix via inversion. Do NOT vortex or sonicate.
    c. Aliquot 90 µL into 1.5 mL tubes. Store at 2˚C for 6-8 weeks –20˚C for 9 months.
4. Buffer RLT (provided with kit) is not used in this protocol; thus, you do not need to add ß-ME to it.

NOTE: Steps 12 and forward are taken directly from the Qiagen manual (tissues section).

PROCEDURE

1. Collect necessary materials.
    a. Qiagen RNAeasy Micro Kit (74004)
    b. Samples (usu. Brain punches)
    c. Dry Ice
    d. 70% Ethanol**
    e. 80% Ethanol**
    f. Trizol (Invitrogen; 100 mL; 15596-026)
    g. Chloroform (Sigmal 500 mL; C-2432)
    h. RNAse free Pellet Pestle (Kimball-Kontes; 100/pk; 749521-1590)
    i. Mini-Pestle Mixer/Vibrator
    j. DNase aliquot (in being done).
2. Place samples on dry ice. Because of the speed necessary for some steps, doing more than 8 samples at 1 time is NOT recommended.
3. Add 200 µL Trizol a sample and let thaw at RT for 30 seconds.
4. Homogenize tissue using mini-pestle and vibrator. Avoid generating excessive bubbles and change mini-pestle between groups.
5. Repeat 3/4 until all samples are homogenized.
6. Add 600 µL Trizol to each sample and let stand **4 - 5 MINUTES.**
7. Add 160 µL of chloroform to each tube. Shake vigorously for **15 seconds**.
8. Incubate at RT for **3 MINUTES**.
9. Spin samples at 12000g for **15 MINUTES** at **4˚C.**
10. Label new tubes.
11. Remove the top (clear) layer from the samples and place in new tubes. Note the maximum volume would be 480 µL, though 300-350 µL is more reasonable. Because you will have to add a 1 volume of 70% ethanol in the next step and the column max is 700 µL, using 350 µL of the homogenized lysate is highly recommended.
12. Add 1 volume of 70% EtOH to the tube. Mix with the pipettor. Some precipitate may form.
13. Apply the sample, including any precipitate that may have formed, to an RNeasy MinElute Spin Column in a 2 mL collection tube. Close and centrifuge **15 SECONDS** at ≥ 8000g. Discard flow-through.
14. Add 350 µL of buffer RW1 to the column. Close the tube and centrifuge **15 SECONDS** at ≥ 8000g. Discard the flow-through. Note: if skipping DNAse treatment, use 700 µL and then move to step 18.
15. Remove 1 aliquot of DNase (90 µL). Add 630 µL of buffer RDD. Mix via inversion.
16. Pipet 80 µL of the DNase onto the column. Incubate **15 MINUTES.**
17. Pipet 350 µL of RW1 into the column. Close and centrifuge **15 SECONDS** at ≥ 8000g. Discard flow-through. Also discard the collection tube.
18. Place the column in a new 2 mL collection tube. Add 500 µL of RPE buffer to each column. Close and centrifuge **15 SECONDS** at ≥ 8000g. Discard flow-through.
19. Add 500 µL of 80% EtOH to column. Close and centrifuge **2 MINUTES** at ≥ 8000g to dry the membrane. Discard the flow-through and the collection tube.
20. Transfer to column to a new 2 mL collection tube. Open the cap and spin at full speed for **5 MINUTES** to remove trace EtOH.
21. To elute, add 14 µL of RNase/DNase free water. Place directly on the silica membrane. Spin at max speed for **1 MINUTE** to elute.
22. Read 230, 260, 280, ratios, and concentrations on NanoDrop.