Total RNA Extraction from Animal Tissues using the QIAGEN RNeasy Mini Kit (Darlington Lab)

Important notes before starting

- **Use appropriate amount of tissue. For tissues larger than the weight range for the Mini Kit, use the Midi or Maxi Kit.**
- Weigh tissue (Subtract weight of Eppendorf tube) and find out the appropriate volume of Buffer RTL (Count backward). If the tissue is too large, subdivide into smaller pieces using a mortar and pestle under liquid nitrogen.
- To process, do not allow tissue to thaw (e.g. during weighing) prior disruption in Buffer RLT.
- β-Mercaptoethanol must be added to Buffer RLT.
  Add 10 µL β -ME per 1 mL of Buffer RTL. The solution is stable for 1 month.

Protocol

1. Disrupt and homogenize the sample immediately using Buffer RLT (β-ME added) and a Polytron for 40 sec.

Starting material	Volume of Buffer RLT
Up to 20 mg	350 µL
20 to 30 mg or tissue is difficult to lyse	600 µL

2. Centrifuge lysate for 3 min at maximum speed in a microcentrifuge, and transfer the supernatant into a new Eppendorf tube.

3. Add 1 volume (350 or 600 µL) of 70% ethanol to the cleared lysate, and mix well by pipetting.

4. Apply 700 µL of the sample to an RNeasy mini spin column sitting in a 2-mL collection tube.

5. Centrifuge for 15 sec at maximum speed. Discard flow-through, and reuse the collection tube.

6. Apply aliquots successively onto the RNeasy column and centrifuge as above. Discard flow-through and reuse the collection tube.

7. Pipet 700 µL Buffer RW1 onto the RNeasy column, incubate RNeasy column for 5 min, and centrifuge for 15 sec at maximum speed. Discard flow-through and the collection tube.

8. Transfer RNeasy column to a new 2-mL collection tube.

9. Pipet 500 µL Buffer RPE onto RNeasy column, and centrifuge for 15 sec at maximum speed to wash. Discard flow-through and reuse the collection tube.

10. Pipet 500 µL Buffer RPE onto RNeasy column, and centrifuge for 2 min at maximum speed to dry the RNeasy membrane. Discard flow-through and the collection tube.

    Note: Residual ethanol may interfere with subsequent reaction. Remove the RNeasy column so that the column does not contact the flow-through as this will result in carryover of ethanol.

11. (Optional): Place the RNeasy spin column in a new 2-mL collection tube, and discard the old collection tube with filtrate. Centrifuge at full speed for 1 min.

12. Transfer RNeasy column into a new 1.5-mL Eppendorf tube (remove the lid) and pipet 30 µL of DEPC water directly onto the RNeasy membrane. Centrifuge for 1 min at maximum speed to elute.

13. Pipet 30 µL of DEPC water successively directly onto the RNeasy membrane. Centrifuge for 5 min at maximum speed to elute.

14. Transfer elute to a new Eppendorf tube.

Quantitation of total RNA

Water used for spectrophotometric measurement of RNA should have pH > 7.5. Acidic pH affects the UV absorption spectrum of RNA and significantly decreases its A_{260}/A_{280} ratio. Typically, distilled water has pH < 6. Adjust water to a slightly alkaline pH by adding concentrated Na_2HPO_4 solution to a final concentration of 1 mM.
Pure RNA has an A_{260}/A_{280} ratio of 1.8-2.1 in pH 7.5.

1. Quantitate RNA by diluting 2 µL in 98 µL 1 mM Na_2HPO_4 (1:50 dilution). Absorbance readings at 260 nm should be greater than 0.15 to ensure significance.

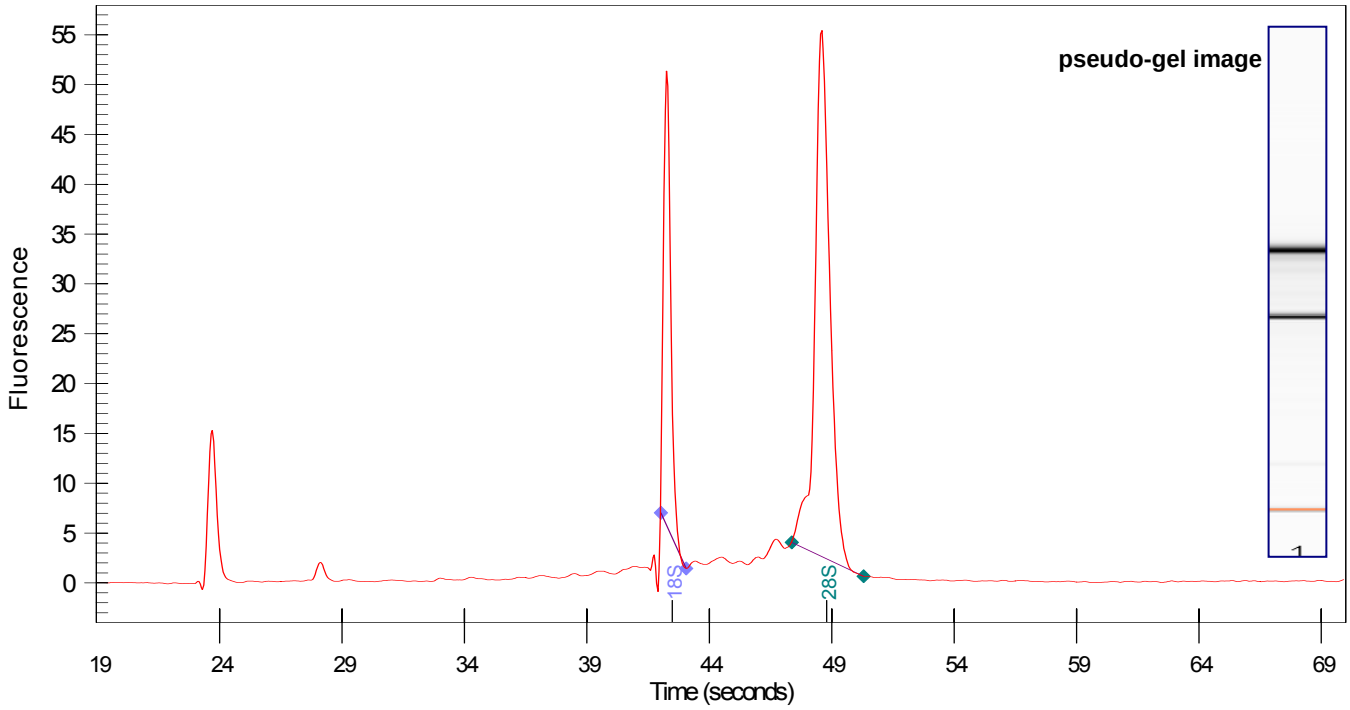
2. An absorbance of 1 unit at 260 nm corresponds to 40 µg of RNA per mL.

Personal communication from QIAGEN

I just wanted to bring one important note to your attention. We at QIAGEN have found that with liver samples only, the ethanol added after RLT should be reduced to 1 volume of 50% ethanol instead of 70% ethanol. We have observed, as have many other customers have, that the high glycogen content of liver results in a precipitation of the nucleic acids after addition of ethanol. Many people had even higher yields when the ethanol was reduced to 50%. Since it worked fine for you with 70% ethanol, you may want to continue that way. It may be worthwhile to try one or two samples with 50% ethanol to see if your yields are comparable or higher then before.

Results from Agilent 2100 Bioanalyzer, RNA Nano Chip, Total RNA

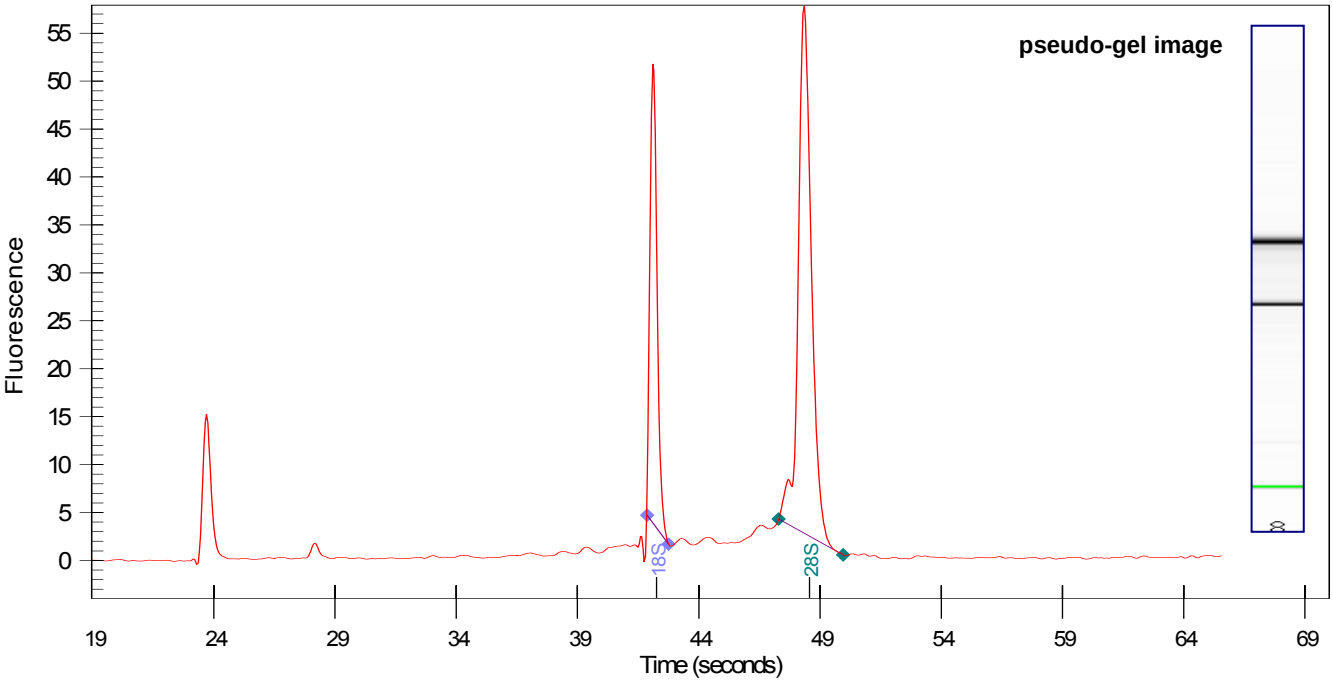
These are results from a Sample Quality Check on RNA extracted using this Extraction Protocol:



BioSizing_Total-RNA-Nano_01504_2002-12-05_14-48-31 sample name: **WT 1 A**

Fragment	Name	Start_Time(secs)	End_Time(secs)	Area	%_of_total_Area
1	18S	42.02	43.05	37.27	19.98
2	28S	47.37	50.30	77.59	41.60

RNA Area	186.51
RNA Concentration(ng/ul)	103.92
rRNA Ratio [28S / 18S]	2.08



BioSizing_Total-RNA-Nano_01504_2002-12-05_14-48-31 sample name: **WT 2 B**

Fragment	Name	Start_Time(secs)	End_Time(secs)	Area	%_of_total_Area
1	18S	41.87	42.75	35.60	20.86
2	28S	47.30	49.95	71.87	42.11

RNA Area	170.70
RNA Concentration(ng/ul)	95.11
rRNA Ratio [28S / 18S]	2.02