SOP: Purification of Total RNA from Animal and Human Tissues using the Qiagen RNeasy mini Kit

Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.

Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

®-Mercaptoethanol (®-ME) must be added to Buffer RLT before use. Add 10 µl ®-ME per 1 ml Buffer RLT. Buffer RLT containing ®-ME can be stored at room temperature (15–25°C) for up to 1 month

Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

The procedure also requires 70% ethanol, which can be prepared by diluting ethanol (96–100%) with distilled water.

Buffer RLT may form a precipitate during storage. If necessary, re-dissolve by warming, and then place at room temperature.

Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. **Do not vortex.** For long-term storage of reconstituted DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Disruption and homogenization using the QIAgen TissueRuptor

Place the tissue in a suitably sized vessel. Add 600 µl Buffer RLT.
Generally, round-bottomed tubes allow more efficient disruption and homogenization than conical-bottomed tubes.

Place the tip of the disposable probe into the vessel and operate the TissueRuptor at full speed until the lysate is homogeneous (40 s).

Note: Make sure the tip of the probe remains submerged in the buffer. Foaming may occur during homogenization. If this happens, let the homogenate stand at room temperature for 2–3 min until the foam subsides before continuing with the procedure.

Centrifuge the lysate for 3 min at full speed. Carefully transfer the supernatant to a new microcentrifuge tube by pipetting. Use only this supernatant (lysate) in subsequent steps. In some preparations, very small amounts of insoluble material will be present after the 3 min centrifugation, making the pellet invisible.

Add 1 volume (usually 600 µl) of 70% ethanol to the lysate, and mix well by pipetting. Do not centrifuge. Note: The volume of 70% ethanol to add may be less than 350 µl if some lysate was lost during homogenization. **Note**: Precipitates may be visible after addition of ethanol, but this does not affect the procedure.

Transfer up to 600 ul of the sample (if volume exceeds 700 ul, centrifuge successive aliquots in the same column) including any precipitate that may have formed, to an RNeasy MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at 8000 x g (10,000 rpm). Discard the flow through. Reuse the collection tube.

Make a DNase I Incubation mix by adding 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube. Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.

Add the DNase I incubation mix (80 µl) directly to the RNeasy spin column membrane, and place on the benchtop (20–30°C) for 15 min. Note: Be sure to add the DNase I incubation mix directly to the RNeasy spin

column membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

Add 350 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at 8000 x g (10,000 rpm) to wash the spin column membrane. Discard the flow-through.* Reuse the collection tube. **Optional**: If on-column DNase digestion is not desired, add 700 µl Buffer RW1 instead, centrifuge for 15 s at 8000 x g, and discard the flow-through and collection tube.

Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at 8000 x g (10,000 rpm) to wash the spin column membrane. Discard the flow-through. Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").

Add 500 µl Buffer RPE to the spin column. Close the lid gently, and centrifuge for 2 min at 8000 x g (10,000 rpm) to wash the spin column membrane. Discard the flow-through.

Place the RNeasy spin column in a new 2 ml collection tube (supplied). Open the lid and centrifuge for 1 min at 8000 x g (10,000 rpm)

Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA. As little as 10 µl RNase-free water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%. Do not elute with less than 10 µl RNase-free water, as the spin column membrane will not be sufficiently hydrated.