February 2021

Quick-Start Protocol

RNeasy® 96 Universal Tissue Kit

QIAzol Lysis Reagent can be stored at room temperature or at 2–8°C and is stable for at least 12 months under these conditions. All other reagents and components of the RNeasy 96 Universal Tissue Kit should be stored at room temperature (15–25°C) and are stable for at least 9 months under these conditions.

Further information

- *RNeasy 96 Universal Tissue Handbook*: **www.qiagen.com/HB-0484**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for purification of total RNA from animal tissues using the vacuum/spin technology.
- QIAzol® Lysis Reagent and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach.
- Add ethanol (96–100%) to Buffer RWT and Buffer RPE as indicated on the bottle label.
- All centrifugation steps are performed in a Centrifuge 4-15C or Centrifuge 4K15C and a vacuum source capable of generating a vacuum pressure of –800 to –900 mbar. The step 9 and the centrifugation step 12 should be done at 4°C. All other steps should be performed at room temperature (15–25°C). Avoid interruptions during the procedure
- Check that all buffers are at room temperature (15–25°C).

Using vacuum/spin technology

1. Assemble the QIAvac 96 vacuum manifold: First, place the waste tray inside the QIAvac base, then place the QIAvac 96 top plate squarely over the QIAvac base. Place an RNeasy® 96 plate in the QIAvac 96 top plate, making sure that the plate is seated tightly. Attach the vacuum manifold to a vacuum source. Keep the vacuum switched off.
2. Disrupt and homogenize flash-frozen or stabilized tissue in 750 µl QIAzol on the TissueLyser for 2x5 min at 25 Hz.

   Do not use more than 50 mg flash-frozen tissue, 25 mg liver, thymus, spleen, or RNAprotect® Tissue Reagent stabilized tissue, or 100 mg adipose tissue.
3. Place the collection microtube rack containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.
4. Load the collection microtube rack into the holder, and place it in the rotor bucket. Centrifuge at 6000 x *g* for 1 min at 4°C to collect residual liquid from the caps of the tubes.
5. Add 150 µl chloroform. Securely cap the collection microtube rack containing the homogenates using new strips of collection microtube caps, and shake it vigorously while inverting the rack for 15 s.
6. Place the collection microtube rack on the benchtop at room temperature for 2–3min.
7. Centrifuge at 6000 x *g* for 15 min at 4°C.
8. Transfer all of the upper, aqueous phases to a new square-well block. Then add 1 volume (usually 400 µl) of 70% ethanol mix by pipetting up and down. Do not centrifuge.
9. Pipet the samples (approx. 800 µl) from step 13 into the wells of the RNeasy 96 plate, and switch on the vacuum source. Apply vacuum until transfer is complete (1–5 min). Switch off the vacuum, and ventilate the QIAvac 96 manifold.

   Note: Tape unused wells with adhesive tape.
10. Add 800 µl of Buffer RW1 to each well of the RNeasy 96 plate. Switch on the vacuum source, and apply vacuum until transfer is complete (1–5 min). Switch off the vacuum, and ventilate the QIAvac 96 manifold.

11. Lift the top plate carrying the RNeasy 96 plate off the base, and empty the waste tray. Reassemble the QIAvac 96 vacuum manifold.
12. Add 800 µl of Buffer RPE to each well of the RNeasy 96 plate, and switch on the vacuum source. Apply vacuum until transfer is complete (1–5 min). Switch off the vacuum, and ventilate the QIAvac 96 manifold.
13. Place the RNeasy 96 plate on top of a square-well block.
14. Add another 800 µl of Buffer RPE to each well of the RNeasy 96 plate. Seal the RNeasy 96 plate with an AirPore Tape Sheet. Load the square-well block and RNeasy 96 plate into the holder, and place the whole assembly in the rotor bucket. Centrifuge at 6000 rpm (approx. 5600 x *g*) for 10 min at room temperature to dry the plate membranes.
15. Remove the AirPore Tape Sheet. Place the RNeasy 96 plate on top of a clean elution microtube rack containing 1.2 ml elution microtubes.
16. To elute the RNA, add 45–70 µl RNase-free water to each well, and seal the RNeasy 96 plate with a new AirPore Tape Sheet. Incubate for 1 min at room temperature. Then centrifuge at 6000 rpm (approx. 5600 x *g*) for 4 min at room temperature.

    Note: Make sure to pipet the RNase-free water directly onto the RNeasy membrane. Elution will be incomplete if some of the water sticks to the walls or the O-rings of the RNeasy 96 plate.
17. Remove the AirPore Tape Sheet. Repeat the elution step (step 21) once with a second volume of 45–70 µl RNase-free water.

    Note: Repeating the elution step is required for complete recovery of RNA. Use elution microtube caps (caps for strips) provided to seal the microtubes for storage. Store RNA at –20 or –70°C.

Document Revision History

Date	Changes
02/2021	Initial release

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.