April 2019

Quick-Start Protocol

RNeasy® 96 Kit

All other reagents and components of the RNeasy 96 Kits (cat. nos. 74181 and 74182) should be stored at room temperature (15–25°C).

Further information

- *RNeasy 96 Handbook*: **www.qiagen.com/HB-2045**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for the purification of total RNA from 5 x 10^5 cells using the vacuum/spin technology.
- All centrifugation steps are performed in a Centrifuge 4-16S (cat. no. 81510) or Centrifuge 4-16KS (cat. no. 81610) and a vacuum source capable of generating a vacuum pressure of –800 to –900 mbar.
- Buffer RLT may form a precipitate upon storage. If necessary, warm to 37°C to redissolve.
- When isolating RNA from cells containing high amounts of RNases, it may be necessary to add β-mercaptoethanol (β-ME) to Buffer RLT to avoid degradation of RNA. β-ME supports the inactivation of RNases by GITC. Add 10 µl of 14.5 M β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME. In most cases, it will not be necessary to add β-ME to Buffer RLT.

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) to obtain a working solution.
- All steps of the RNeasy 96 protocol for isolation of total RNA should be performed at room temperature (15–25°C). Avoid interruptions during the procedure.

1. Preparing the QIAvac 96 vacuum manifold: Place the waste tray inside the QIAvac base. Place the top plate squarely over the base. Place the RNeasy 96 plate into the QIAvac top plate, making sure that the plate is seated tightly. Attach QIAvac 96 manifold to the vacuum source. Keep vacuum switched off.

   Note: When placing the RNeasy 96 plate into the vacuum manifold, make sure that the beveled edges point to the right-hand side.
2. Harvest a maximum of 5×10^5 cells, as a cell pellet or direct lysis in the vessel.
3. Add 150 µl of Buffer RLT into each well of the microplate. Keeping the microplate flat on the bench, shake the microplate vigorously back and forth for 10 s. Continue to keep the plate flat on the bench as you rotate the plate by 90°, and then shake the microplate again for an additional 10 s.

   Note: After the addition of ethanol in step 4, total volume will be 300 µl.
4. Add 1 volume (150 µl) of 70% ethanol. Mix by pipetting up and down 3 times.
5. Apply the samples (300 µl) from step 4 into the wells of the RNeasy 96 plate, and then switch on the vacuum source. Apply vacuum until transfer is complete (15–60 s). Switch off vacuum, and ventilate the QIAvac 96 manifold.

   The flow-through is collected in the waste tray.

   Note: Tape unused wells with adhesive tape. Do not use the AirPore Tape Sheets supplied with the RNeasy 96 Kit. Use either adhesive tape or Tape Pads (cat. no. 19570) from QIAGEN.
6. DNase digestion (optional): Pipet 80 µl of the DNase I incubation mix directly onto the RNeasy membrane in each well of the RNeasy 96 plate. Seal the plate with an AirPore Tape Sheet. Place at room temperature for 15 min. Remove the AirPore Tape from the RNeasy 96 plate. Proceed to step 7.

7. Add 1 ml of Buffer RW1 to each well of the RNeasy 96 plate. (If on-membrane DNase digestion was performed in the previous step, wait 5 min before proceeding.) Switch on vacuum source and apply vacuum until transfer is complete (10–30 s). Switch off vacuum and ventilate QIAvac 96 manifold.

   Note: To efficiently remove DNase following DNase digestion, allow Buffer RW1 to remain on the RNeasy membrane for 5 min before vacuuming the buffer through the membrane. Collect wash fraction in the same waste tray used in step 5.

8. Lift the top plate carrying the RNeasy 96 plate from the base, and empty the waste tray. Reassemble the QIAvac 96 vacuum manifold.
9. Add 1 ml of Buffer RPE to each well of the RNeasy 96 plate and switch on vacuum source. Apply vacuum until transfer is complete (10–30 s). Switch off vacuum and ventilate QIAvac 96 manifold.
10. Place the RNeasy 96 plate on top of a square-well block. Mark the RNeasy plate for later identification.
11. Add another 1 ml 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 (~5600 x *g*) for 10 min at room temperature to dry the plate membranes.

    Centrifugation with sealed plates prevents cross-contamination.

    It is important to dry the RNeasy membrane, because residual ethanol may interfere with subsequent reactions. The 10 min spin ensures that residual traces of salt are removed and that no ethanol is carried over during elution.

12. Remove the AirPore Tape. Place RNeasy 96 plate on top of an elution microtube rack containing 1.2 ml elution microtubes.

13. To elute the RNA, add 45–70 µl RNase-free water to each well, and seal the RNeasy 96 plate with a new sheet of AirPore Tape. Incubate for 1 min at room temperature, and then centrifuge at 6000 rpm (~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.

14. Remove the AirPore Tape. Repeat the elution step (step 13) with a second volume of 45–70 µl RNase-free water.

    Note: Repeating the elution step is required for complete recovery of RNA. The elution volume will be approximately 15 µl less than the volume of RNase-free water added to the membrane, corresponding to the membrane dead volume.

    Use caps provided to seal the microtubes for storage. Store RNA at –20°C or at –70°C.

Scan QR code for handbook.

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

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