RNeasy® 96 QIAcube® HT Kit, Part 2

Store RNeasy 96 plates and buffers at room temperature (15–25°C).

Further information

- *RNeasy 96 QIAcube HT Handbook*: www.qiagen.com/handbooks
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: toll-free 00800-22-44-6000, or www.qiagen.com/contact

Notes before starting

- Refer to part 1 of this protocol before proceeding further.

RNA purification — cell samples

1. Harvest cells and add 140 µl Buffer RLT to each microplate well. Keeping the microplate flat on the bench the entire time, shake it vigorously back and forth for 10 s. Rotate the plate by 90° and shake it for an additional 10 s.
2. Add 140 µl lysed sample to the selected S-Block wells. Place the S-Block in the B1 position of the instrument worktable.

RNA purification — tissue samples

1. Place 5 mm stainless steel beads into the collection microtubes (1 bead per tube), and transfer the collection microtube rack to a box with dry ice.
2. Remove the tissue sample from RNA*later*® RNA Stabilization Reagent, or from cold storage. Do not allow the tissue to thaw before it is placed in QIAzol® Lysis Reagent.
3. Determine the amount of tissue. Do not use more than the amount indicated in *RNeasy 96 QIAcube HT Handbook*. Transfer tissue immediately to a cooled collection microtube.
4. Remove the collection microtube rack from dry ice and immediately pipet 750 µl QIAzol Lysis Reagent into each collection microtube. Close the

collection microtube rack with caps and homogenize on the TissueLyser for 5 min at 25 Hz.

5. Rotate the TissueLyser rack to allow even homogenization, and homogenize for another 5 min at 25 Hz.

6. Place the collection microtube rack on the benchtop at room temperature (15–25°C) for 5 min.

7. Place the collection microtube rack into the holder, and place it in the rotor bucket. Centrifuge at 6000 x *g* for 1 min to collect residual liquid from the caps.

8. Add 150 µl chloroform. Using new caps, securely cap the collection microtube rack containing the homogenates, and shake it vigorously while inverting the rack for 15 s. Thorough mixing is important for subsequent phase separation.

9. Place the collection microtube rack on the benchtop at room temperature (15–25°C) for 2–3 min.

10. Centrifuge at 6000 x *g* for 15 min at 4°C. After centrifugation, the sample separates into phases. Transfer only the upper, aqueous phase containing RNA into the S-Block.

Automated sample processing

1. Transfer the volumes of all reagents into the reagent troughs, close lids, and place them on the indicated positions on the worktable. Start the run immediately and perform the pre-run check. After completing the pre-run check, close the instrument hood and click "OK". Click "Cancel" when the "Save as" dialog box appears. The protocol run begins.

2. Cover the elution plate (EMTR) with the lid, and remove from the elution chamber, when the protocol is complete. Discard used plasticware. We recommend discarding leftover reagents in the reagent troughs. Clean the carriage, channeling block, channeling block holder, and tip chute. Turn on the UV lamp to decontaminate the worktable.