January 2020

Quick-Start Protocol

RNeasy® Plus 96 Kit

Purification of Total RNA from Cells Using Vacuum/Spin Technology

All reagents and components of the RNeasy Plus 96 Kit should be stored at room temperature (15–25°C) and are stable for at least 9 months under these conditions, if not otherwise stated on label.

Further information

- *RNeasy® Plus 96 Handbook*: **www.qiagen.com/HB-2063**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for purification of total RNA from 1 x 10^6 cells using the vacuum/spin technology. For purifying total RNA from cells using the spin technology or for purifying total RNA containing small RNAs from cells using either vacuum/spin or spin technology, refer to the *RNeasy Plus 96 Handbook*.
- All centrifugation steps are performed in a Centrifuge 4-15C or Centrifuge 4K15C with the Plate Rotor 2 x 96.
- All vacuum steps are performed on the QIAvac 96 vacuum manifold.
- Buffer RLT Plus and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach.
- Buffer RLT Plus may form a precipitate upon storage. If necessary, warm to 37°C to redissolve.

- If purifying RNA from cell lines rich in RNases, we recommend adding β-mercaptoethanol (β-ME) to Buffer RLT Plus before use. Add 10 µl β-ME per 1 ml Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus containing β-ME can be stored at room temperature for up to 1 month. Alternatively, add 20 µl of 2M dithiothreitol (DTT) per 1 ml Buffer RLT Plus. The stock solution of 2M DTT in water should be prepared fresh or kept frozen in single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.
- Buffer RPE is supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96-100%) as indicated on the bottle to obtain a working solution.
- All steps in the RNeasy Plus 96 protocol for the isolation of total RNA should be performed at room temperature. Avoid interruptions during the procedure.

Using vacuum/spin technology

1. Harvest cells (up to 1 x 10^6 cells) according to step 1a or 1b.
   1a. Cells grown in a monolayer:
   Completely remove the cell-culture medium by pipetting, and add 300 µl Buffer RLT Plus to each well. Transfer the lysates to a rack of collection microtubes (cat. no. 19560), and seal the tubes with collection microtube caps (cat. no. 19566).
   1b. Cells grown in suspension:
   Transfer up to 1 x 10^6 cells from each sample to a rack of collection microtubes (cat. no. 19560). Pellet the cells by centrifuging for 5 min at 300 x *g*. Completely remove all supernatant by pipetting, and add 300 µl Buffer RLT Plus to each tube. Seal the tubes with collection microtube caps (cat. no. 19566).
2. Homogenize the lysates by vortexing the rack of collection microtubes at full speed for at least 30 s.

3. Place a gDNA Eliminator 96 plate on top of a new S-Block. Mark the plate for later identification.
4. Transfer the lysates from step 2 to the wells of the gDNA Eliminator 96 plate.
5. Seal the gDNA Eliminator 96 plate with an AirPore tape sheet. Place the S-Block and gDNA Eliminator 96 plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C. Discard the gDNA Eliminator 96 plate, and save the flow-through.
6. 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.
7. Add 1 volume (300 µl) of 70% ethanol to each well of the S-Block containing the flow-through from step 5. Mix well by pipetting up and down 3 times.
8. Transfer the samples (600 µl) to the wells of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the samples have completely passed through the membranes (15–60 s). Switch off the vacuum, and ventilate the manifold.
9. Add 800 µl Buffer RW1 to each well of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the buffer has completely passed through the membranes (10–30 s). Switch off the vacuum, and ventilate the manifold.

   Note: The flow-through is collected in the same waste tray from step 8.
10. Lift the QIAvac 96 top plate carrying the RNeasy 96 plate from the QIAvac base, and empty the waste tray. Reassemble the QIAvac 96 vacuum manifold.
11. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the buffer has completely passed through the membranes (10–30 s). Switch off the vacuum, and ventilate the manifold.
12. Place the RNeasy 96 plate on top of an S-Block (either new or reused). Mark the plate for later identification.

13. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and seal the plate with an AirPore tape sheet. Place the S-Block and RNeasy 96 plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (~5600 x *g*) for 10 min at 20–25°C to dry the membranes.
14. Remove the AirPore tape sheet. Place the RNeasy 96 plate on top of a rack of Elution Microtubes CL. Add 45–70 µl RNase-free water to each well, and seal the plate with a new AirPore tape sheet. Incubate for 1 min at room temperature. Then centrifuge at 6000 rpm (~5600 x *g*) for 4 min at 20–25°C to elute the RNA.
15. Remove the AirPore tape sheet. Repeat step 14 with a second volume of 45–70 µl RNase-free water.

Revision History

Date	Changes
01/2020	Initial release

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