June 2023

Quick-Start Protocol

QIAwave® RNA Mini Kit

The QIAwave RNA Mini Kit (cat. no. 74534 and cat. no. 74536) can be stored at room temperature (15–25°C) up to one year after delivery.

Further information

- *QIAwave RNA Mini Handbook:* **www.qiagen.com/HB-2989**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**
- The QIAwave RNA Mini Kit can be automated on the QIAcube Connect using the RNeasy Mini Kit protocols that be downloaded at **www.qiagen.com/qiacubeprotocols**.

Notes before starting

- If purifying RNA from cell lines rich in RNases, or tissue, add either 10 µL β-mercaptoethanol (β-ME), or 20 µL 2 M dithiothreitol (DTT), to 1 mL Buffer RLT. Buffer RLT with β-ME or DTT can be stored at room temperature for up to 1 month.
- Remove RNAprotect® Tissue Reagent stabilized tissue from the reagent using forceps.
- Preassemble RNeasy® Mini spin columns with Waste Tubes (provided).
- **Preparation of final buffer from buffer concentrates**: Transfer the entire volume of the buffer concentrate from the 2 mL tube or 15 mL bottle into a glass bottle appropriate for the final volume (Table 1), either by using a pipette or by pouring. Add ultrapure water and/or ethanol (96–100%) according to Table 1. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.

Table 1. Preparation of final buffers from buffer concentrates

Kit (cat. no.)	Final buffer	Buffer concentrate*	Ultrapure water	Ethanol (96–100%)	Final volume
74534	RPE	RPE/C	12 mL	52 mL	65 mL
74536	RPE	RPE/C	60 mL	260 mL	325 mL

*Use entire volume.

Procedure

1. **Cells**: Harvest a maximum of 1 x 10^7 cells, as a cell pellet or by direct lysis in the vessel. Add the appropriate volume of Buffer RLT and select a suitable method for disruption and homogenization (see Table 2).

   Tissues: Do not use more than 30 mg tissue. Disrupt the tissue and homogenize the lysate in the appropriate volume of Buffer RLT (see Table 2). Centrifuge the lysate for 3 min at maximum speed. Carefully remove the supernatant by pipetting, and use it in step 2.

Table 2. Volumes of Buffer RLT for sample disruption and homogenization

Sample	Amount	Dish	Buffer RLT	Disruption and homogenization
Animal cells	<5 x 10^6 ≤1 x 10^7	<6 cm 6–10 cm	350 µL 600 µL	Add Buffer RLT, vortex (≤1 x 10^5 cells); or use QIAshredder, TissueRuptor II®, or needle and syringe
Animal tissues	<20 mg ≤30 mg	– –	350 µL * 600 µL	TissueLyser LT, TissueLyser II, TissueLyser III, TissueRuptor II, or mortar and pestle followed by QIAshredder or needle and syringe

* Use 600 µL Buffer RLT for tissues stabilized in RNAprotect Tissue Reagent, or for difficult-to-lyse tissue

2. Add 1 volume of 70% ethanol to the lysate, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 3.
3. Transfer up to 700 µL of the sample, including any precipitate, to a RNeasy Mini spin column placed in a 2 mL Waste Tube (supplied). Close the lid, and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through and reuse the Waste Tube.

Optional: For DNase digestion, follow steps 1–4 of "Appendix D: Optional On Column DNase Digestion with the RNase Free DNase" in the *QIAwave RNA Mini Handbook*.

4. Add 700 µL Buffer RW1 to the RNeasy spin column. Close the lid, and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through and reuse the Waste Tube.
5. Add 500 µL Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through and reuse the Waste Tube.
6. Add 500 µL Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 2 min at ≥8000 x *g*. Discard the flow-through.

   Optional: Place the RNeasy Mini spin column back into the same Waste Tube, centrifuge at full speed for 1 min to dry the membrane. Discard the Waste Tube.
7. Place the RNeasy Mini spin column in a new 1.5 mL microcentrifuge tube (not supplied). Add 30–50 µL RNase-free water directly to the spin column membrane. Close the lid, and centrifuge for 1 min at ≥8000 x *g* to elute the RNA.
8. If the expected RNA yield is >30 µg, repeat step 7 using another 30–50 µL of RNase-free water, or using the eluate from step 7 (if high RNA concentration is required). Reuse the microcentrifuge tube from step 7.

Document Revision History

Date	Changes
01/2022	Initial release
05/2023	Added cat. no. 74534 and necessary procedures. Edited according to new brand template.

Scan QR code for handbook.

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