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Quick-Start Protocol

QIAwave RNA Mini Kit

The QIAwave RNA Mini Kit (cat. no. 74536) can be stored at room temperature (15–25°C) for up to 1 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**

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.
- Preparation of Buffer RPE/C: Transfer the entire volume of Buffer RPE/C from the 15 ml bottle into a glass bottle larger than 325 ml, either by using a pipet or by pouring. Add 60 ml ultrapure water such as nuclease-free water (1000 ml, cat. no.129115; 5 liters, cat. no. 129117) and 260 ml ethanol (96–100%) to obtain a final volume of 325 ml. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, peel off the upper label from the piggyback label on the 15 ml plastic bottle and transfer it onto the glass bottle.
- Remove RNAprotect® Tissue Reagent-stabilized tissue from the reagent using forceps.
- Preassemble RNeasy® Mini spin columns with Waste Tubes.

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 (see Table 1).

   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 1). Centrifuge the lysate for 3 min at maximum speed. Carefully remove the supernatant by pipetting, and use it in step 2.

Table 1. Volumes of Buffer RLT for sample disruption and homogenization

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

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

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 Mini 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/C to the RNeasy Mini 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/C to the RNeasy Mini 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 collection tube from step *7*.

Document Revision History

Date	Changes
01/2022	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.