

## Quick-Start Protocol

# QIAwave RNA Plus Mini Kit

The QIAwave RNA Plus Mini Kit (cat. nos. 74634 and 74636) can be stored at room temperature (15–25°C) for at least 12 months if not otherwise stated on label.

### Further information

- *QIAwave RNA Plus Mini Kit Handbook*: [www.qiagen.com/HB-3136](http://www.qiagen.com/HB-3136)
- Safety Data Sheets: [www.qiagen.com/safety](http://www.qiagen.com/safety)
- Technical assistance: [support.qiagen.com](http://support.qiagen.com)

### Notes before starting

- If purifying RNA from cell lines rich in RNases, or tissue, add either 10  $\mu$ L  $\beta$ -mercaptoethanol ( $\beta$ -ME), or 20  $\mu$ L 2 M dithiothreitol (DTT), to 1 mL Buffer RLT Plus. Buffer RLT Plus with  $\beta$ -ME or DTT can be stored at room temperature for up to 1 month.
- Foaming can be reduced by adding Reagent DX (cat. no. 19088) at a final concentration of 0.5% (v/v) before disruption and homogenization.
- Preassemble RNeasy® Mini spin columns with Waste Tubes.
- **Preparation of final buffers 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 buffer from buffer concentrates**

Kit (cat. no.)	Final buffer	Buffer concentrate*	Ultrapure water	Ethanol (96-100%)	Final volume
74634	RPE	RPE/C	12 mL	52 mL	65 mL
74636	RPE	RPE/C	60 mL	260 mL	325 mL

\*Use entire volume

## Procedure

1. **Cells:** Harvest a maximum of  $1 \times 10^7$  cells, as a cell pellet or by direct lysis in the vessel. Add the appropriate volume of Buffer RLT Plus (see Table 2). Vortex for 30 s, or homogenize.

**Tissues:** Disrupt tissue ( $\leq 30$  mg) and homogenize the lysate in the appropriate volume of Buffer RLT Plus (see Table 2). Centrifuge the lysate for 3 min at maximum speed. Carefully remove the supernatant by pipetting and use it in step 2.

2. Transfer the homogenized lysate to a gDNA Eliminator spin column placed in a 2 mL collection tube (supplied).

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

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

\* Use 600  $\mu$ L Buffer RLT Plus for tissues stabilized in RNAlater® Tissue, or for difficult-to-lyse tissues.

3. Centrifuge for 30 s at  $\geq 8000 \times g$  ( $\geq 10,000$  rpm). Discard the column, and save the flow through. Add 1 volume (usually 350 or 600  $\mu$ L) of 70% ethanol to the flow through, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 4.

4. Transfer up to 700  $\mu\text{L}$  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  $\geq 8000 \times g$ . Discard the flow through and re-use the Waste Tube.
5. Add 700  $\mu\text{L}$  Buffer RW1 to the RNeasy spin column. Close the lid, and centrifuge for 15 s at  $\geq 8000 \times g$ . Discard the flow through and re-use the Waste Tube.
6. Add 500  $\mu\text{L}$  Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 15 s at  $\geq 8000 \times g$ . Discard the flow through and re-use the Waste Tube.
7. Add 500  $\mu\text{L}$  Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 2 min at  $\geq 8000 \times g$ . Discard the flow through.

**Optional:** Place the RNeasy spin column back into the same Waste Tube, centrifuge at full speed for 1 min to dry the membrane.

8. Place the RNeasy spin column in a new 1.5 mL microcentrifuge tube (not supplied). Add 30–50  $\mu\text{L}$  RNase-free water directly to the spin column membrane. Close the lid, and centrifuge for 1 min at  $\geq 8000 \times g$  to elute the RNA.

**Optional:** Repeat elution with another volume of water or with RNA eluate.

## Document Revision History

Date	Changes
05/2023	001: Initial release; 002: Revised preparation to final buffers from buffer concentrates.



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