Quick-Start Protocol May 2023

QIAwave® DNA/RNA Mini Kit

The QIAwave DNA/RNA Mini Kit (cat. no. 80504) can be stored at room temperature (15–25°C) for at least 12 months if not otherwise stated on label.

The QIAwave DNA/RNA Mini Kit purifies genomic DNA and total RNA simultaneously from a single sample. Lysate from homogenized cells or tissue is first passed through an AllPrep® DNA spin column to isolate DNA, then through a RNeasy® spin column to isolate RNA.

Further information

- *QIAwave DNA/RNA Mini Kit Handbook*: **www.qiagen.com/HB-3138**
- 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 Plus. Buffer RLT Plus with β-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.
- **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 buffers from buffer concentrates

Kit (cat. no.)	Final buffer	Buffer*	Ultrapure water	Ethanol (96–100%)	Final volume
80504	RPE	RPE/C	12 mL	52 mL	65 mL
	AW1	AW1/C	–	20 mL	35 mL
	AW2	AW2/C	15 mL	40 mL	56.5 mL
	EB	EB/C	20 mL	–	22 mL

*Use entire volume.

Sample disruption and homogenization of cells or tissue

1. **Cells**: Harvest a maximum of 1 x 10^7 cells, as a cell pellet or by direct lysis in the cell-culture dish (up to 10 cm diameter). Add the appropriate volume of Buffer RLT Plus and homogenize (see Table 2).

   Tissues: Disrupt tissue (≤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.

2. Transfer the homogenized lysate to an AllPrep DNA spin column placed in a 2 mL collection tube (supplied). Close the lid gently, and centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm).

3. Use the flow through for RNA purification.

4. Place the AllPrep DNA spin column in a new 2 mL Waste Tube (supplied). Store at room temperature or at 4°C for later DNA purification.

   Note: Do not store the column at room temperature, or 4°C, for long periods. Do not freeze the column.

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

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

* Use 600 µL Buffer RLT Plus for tissues stabilized in RNAprotect® Tissue, or for difficult-to-lyse tissues.

† For optimal DNA yields, thorough homogenization is required (e.g., by TissueRuptor, TissueLyser LT, TissueLyser II or TissueLyser III).

Total RNA purification

5. Add 1 volume of 70% ethanol to the flow through from step 2. Mix well by pipetting. Do not centrifuge. Proceed immediately to step 6.
6. Transfer up to 700 µL of the sample, including any precipitate, to the RNeasy spin column placed in a 2 mL Waste Tube (supplied). Centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow through.

   Note: Reuse this Waste Tube through steps 7, 8, and 9.
7. 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.
8. 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.
9. 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 spin column back into the same Waste Tube. Centrifuge at full speed for 1 min to dry the membrane. Discard the Waste Tube.
10. Place the RNeasy 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.

Optional: If the expected RNA yield is >30 µg, repeat step 10 using another 30–50 µL of RNase-free water, or using the eluate from step 10 (if high RNA concentration is required). Reuse the microcentrifuge tube from step 10.

Genomic DNA purification

11. Add 500 µL Buffer AW1 to the AllPrep DNA spin column (in 2 mL Waste Tube) from step 3. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow through. Reuse the Waste Tube in step 12.
12. Add 500 µL Buffer AW2 to the AllPrep DNA spin column. Close the lid gently, and centrifuge for 2 min at full speed to wash the spin column membrane.
13. Place the AllPrep DNA spin column in a new 1.5 mL microcentrifuge tube (not supplied). Add 100 µL Buffer EB directly to the spin column membrane and close the lid. Incubate at room temperature for 1 min. Centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the DNA.

    Optional: Repeat step 13 using another 100 µL of Buffer EB, or using the eluate from step 13 (if higher DNA concentration is required). Reuse the microcentrifuge tube from step 13.

Document Revision History

Date	Changes
05/2023	Initial release

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