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

- 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.
- 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

<table>
<thead>
<tr>
<th>Kit (cat. no.)</th>
<th>Final buffer</th>
<th>Buffer concentrate*</th>
<th>Ultrapure water</th>
<th>Ethanol (96-100%)</th>
<th>Final volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>74634</td>
<td>RPE</td>
<td>RPE/C</td>
<td>12 mL</td>
<td>52 mL</td>
<td>65 mL</td>
</tr>
<tr>
<td>74636</td>
<td>RPE</td>
<td>RPE/C</td>
<td>60 mL</td>
<td>260 mL</td>
<td>325 mL</td>
</tr>
</tbody>
</table>

*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.

2. **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.

3. 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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount</th>
<th>Dish</th>
<th>Buffer RLT Plus (μL)</th>
<th>Disruption and homogenization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelleted cells</td>
<td>&lt;5 x 10⁶</td>
<td>&lt;6 cm</td>
<td>350</td>
<td>Add Buffer RLT Plus, vortex ($\leq 1 \times 10^5$ cells); or use QIAshredder, TissueRuptor®, TissueRuptor II, or needle and syringe</td>
</tr>
<tr>
<td></td>
<td>$\leq 1 \times 10^7$</td>
<td>6–10 cm</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>Animal tissues</td>
<td>&lt;20 mg</td>
<td>–</td>
<td>350*</td>
<td>TissueLyser LT, TissueLyser II, TissueLyser III, TissueRuptor, TissueRuptor II, or mortar and pestle followed by QIAshredder or needle and syringe</td>
</tr>
<tr>
<td></td>
<td>$\leq 30$ mg</td>
<td>–</td>
<td>600</td>
<td></td>
</tr>
</tbody>
</table>

* Use 600 μL 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 μL) 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 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 re-use the Waste Tube.

5. 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 re-use the Waste Tube.

6. 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 re-use the Waste Tube.

7. 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.

8. 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:** Repeat elution with another volume of water or with RNA eluate.
## Document Revision History

<table>
<thead>
<tr>
<th>Date</th>
<th>Changes</th>
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<tbody>
<tr>
<td>05/2023</td>
<td>001: Initial release; 002: Revised preparation to final buffers from buffer concentrates.</td>
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</tbody>
</table>

Scan QR code for handbook.

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"RNAlater®" is a trademark of AMBION, Inc., Austin, Texas and is covered by various U.S. and foreign patents.

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