The RNeasy Mini Kit (cat. nos. 74104 and 74106) can be stored at room temperature (15–25°C) for at least 9 months if not otherwise stated on label.

Further information

- 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 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.
- Add 4 volumes of ethanol (96–100%) to Buffer RPE for a working solution.
- Remove RNAprotect® stabilized tissue from the reagent using forceps.
- For RNeasy Protect Mini Kit (cat. nos. 74124 and 74126), please start with the Quick-Start Protocol RNAprotect Tissue Reagent, RNAprotect Tissue Tubes, and RNeasy Protect Kits.

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 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.

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 an RNeasy Mini spin column placed in a 2 ml collection tube (supplied). Close the lid, and centrifuge for 15 s at ≥8000 x g. Discard the flow-through.
Optional: For DNase digestion, follow steps 1–4 of “On column DNase digestion” in Quick-Start Protocol RNeasy Mini Kit, Part 2.

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.

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.

6. Add 500 μl Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 2 min at ≥8000 x g.

Optional: Place the RNeasy spin column in a new 2 ml collection tube (supplied). Centrifuge at full speed for 1 min to dry the membrane.

7. Place the RNeasy spin column in a new 1.5 ml collection tube (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.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount</th>
<th>Dish</th>
<th>Buffer RLT (μl)</th>
<th>Disruption and homogenization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal cells</td>
<td>&lt;5 x 10⁶</td>
<td>&lt;6 cm</td>
<td>350</td>
<td>Add Buffer RLT, vortex (&lt;1 x 10⁵ cells); or use QIAshredder, TissueRuptor®, or needle and syringe</td>
</tr>
<tr>
<td></td>
<td>&lt;1 x 10⁷</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; TissueRuptor, or mortar and pestle followed by QIAshredder or needle and syringe</td>
</tr>
<tr>
<td></td>
<td>≤30 mg</td>
<td>–</td>
<td>600</td>
<td></td>
</tr>
</tbody>
</table>

Document Revision History

<table>
<thead>
<tr>
<th>Date</th>
<th>Changes</th>
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<tr>
<td>11/2021</td>
<td>Removed reference to RNAlater.</td>
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