

RNA extraction

Cells: Harvest a maximum of 1×10^7 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.

2. Add 1 volume of 70% ethanol to the lysate, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 3.

Sample to Insight

3. Transfer up to 700 μ l 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 $\geq 8000 \times g$. 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 μ 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.
5. Add 500 μ 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.
6. Add 500 μ l Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 2 min at $\geq 8000 \times g$.
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 μ 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.
8. If the expected RNA yield is $>30 \mu\text{g}$, repeat step 7 using another 30–50 μ l of RNase-free water, or using the eluate from step 7 (if high RNA concentration is required). Reuse the collection tube from step 7.