RNeasy® Mini Kit, Part 2

The RNeasy Mini Kit (cat. nos. 74104 and 74106) can be stored at room temperature (15–25°C) for at least 9 months.

For more information, additional and more detailed protocols, and safety information, please refer to the *RNeasy Mini Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

On-column DNase digestion

Notes before starting

- If using the RNase-Free DNase Set for the first time, prepare DNase I stock solution by injecting 550 µl RNase-free water into the DNase I vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex.
- For long-term storage of DNase I stock solution, divide it into single-use aliquots and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze aliquots after thawing.

1. Add 350 µl Buffer RW1 to RNeasy column, close lid, centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard flow-through.
2. Add 10 µl DNase I stock solution (see above) to 70 µl Buffer RDD. Mix by gently inverting the tube. Centrifuge briefly.
3. Add DNase I incubation mix (80 µl) directly to RNeasy column membrane, and place on benchtop (20–30°C) for 15 min.
4. Add 350 µl Buffer RW1 to RNeasy column, close lid, centrifuge for 15 s at ≥8000 x *g*. Discard flow-through. Continue with step 5 of "RNA purification from cells/tissue samples" in *Quick-StartProtocol RNeasy Mini Kit, Part 1*, or step 4 of "RNA cleanup" (below).

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RNA cleanup

Notes before starting

- Add 4 volumes of ethanol (96–100%) to Buffer RPE for a working solution.

1. Adjust the sample to a volume of 100 µl with RNase-free water. Alternatively, follow steps in "DNase digestion of RNA before RNA cleanup" in Appendix E of *RNeasy Mini Handbook*. Add 350 µl Buffer RLT, and mix well.
2. Add 250 µl ethanol (96–100%) to the diluted RNA, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 3.
3. Transfer the sample (700 µl) to an RNeasy Mini spin column placed in a 2 ml collection tube (supplied). Close the lid. Centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.

   Optional: If performing optional on-column DNase digestion, follow steps 1–4 of "On-column DNase digestion" (above) after this step.
4. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid. Centrifuge for 15 s at ≥8000 x *g* to wash the membrane. Discard the flow-through.
5. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid. Centrifuge for 2 min at ≥8000 x *g* to wash the membrane.

   Optional: Place the RNeasy spin column in a new 2 ml collection tube (supplied). Close the lid, and centrifuge at full speed for 1 min.
6. 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.
7. If the expected RNA yield is >30 µg, repeat step 6 using another 30–50 µl of RNase-free water. Alternatively, use the eluate from step 6 (if high RNA concentration is required). Reuse the collection tube from step 6.

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