Quick-Start Protocol April 2016

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 if not otherwise stated on label.

Further information

- *RNeasy Mini Handbook*: **www.qiagen.com/HB-0435**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

On-column DNase digestion

- 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-Start Protocol RNeasy Mini Kit, Part 1*, or step 4 of "RNA cleanup" (below).

Notes before starting

RNA cleanup

- 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.

Scan QR code for handbook.

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