Quick-Start Protocol March 2016

RNeasy® Micro Kit

The RNeasy Micro Kit (cat. no. 74004) is shipped at ambient temperature. Store the RNeasy MinElute® spin columns and the RNase-Free DNase Set immediately at 2–8°C. Store the remaining components dry at room temperature (15–25°C). All kit components are stable for at least 9 months under these conditions if not otherwise stated on label.

Further information

- *RNeasy Micro Handbook*: **www.qiagen.com/HB-1920**
- 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 before use. Buffer RLT containing DTT or β-ME 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.
- When processing <500 cells, carrier RNA may be added to the lysate before homogenization (see the *RNeasy Micro Handbook* for information).
- To prepare DNase I stock solution, dissolve the lyophilized DNase I in 550 µl RNase-free water. Mix gently by inverting the vial. Do not vortex. Store DNase I as single-use aliquots at –20°C for up to 9 months, or store at 2–8°C for up to 6 weeks. Do not refreeze after thawing.

1. **Cells**: Harvest a maximum of 5 x 10^5 cells, as a cell pellet, or by direct lysis in the vessel. Add 350 µl Buffer RLT and homogenize.

   Tissues: Disrupt and homogenize ≤5 mg tissue in 350 µl Buffer RLT using the TissueRuptor® or TissueLyser instruments. Centrifuge the lysate for 3 min at maximum speed. Carefully remove the supernatant by pipetting and use it for step 2.

Microdissected cryosections: Collect the sample directly into an appropriate volume of Buffer RLT. (The volume depends on the collection vessel used for microdissection, but should not exceed 65 µl [Leica® instruments] or 300 µl [other instruments].) Adjust the volume to 350 µl with Buffer RLT. Vortex for 30 s.

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 the sample, with any precipitate, to an RNeasy MinElute spin column in a 2 ml collection tube (supplied). Close the lid and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.
4. Add 350 µl Buffer RW1 to the RNeasy MinElute spin column. Close the lid. Centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.
5. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by inverting the tube. Add the DNase I incubation mix (80 µl) directly to the RNeasy MinElute spin column membrane. Place on the benchtop (20–30°C) for 15 min. Add 350 µl Buffer RW1 to the RNeasy MinElute spin column. Close the lid, and centrifuge for 15 s at ≥8000 x *g*. Discard the collection tube.
6. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Add 500 µl Buffer RPE to the spin column. Close the lid, and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.
7. Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid, and centrifuge for 2 min at ≥8000 x *g*. Discard the collection tube.
8. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min to dry the membrane. Discard the flow-through and collection tube.
9. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.

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