Quick-Start Protocol March 2016

RNeasy® Mini Kit, Part 1

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

- 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 RNA*later*® stabilized tissue from the reagent using forceps.
- For RNeasy Protect Mini Kit (cat. nos. 74124 and 74126), please start with the *Quick-Start Protocol RNA*later *RNA Stabilization Reagent, RNAlater TissueProtect Tubes, and RNeasy Protect Kits*.

* This option not included for cells in handbook; handbook to be updated.

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 (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

Sample	Amount	Dish	Buffer RLT	Disruption and homogenization
Animal cells	<5 x 10^6	<6 cm	350 µl	Add Buffer RLT, vortex (≤1 x 10^5 cells); or use QIAshredder, TissueRuptor®, or needle and syringe
	≤1 x 10^7	6–10 cm	600 µl	
Animal tissues	<20 mg	–	350 µl*	TissueLyser LT; TissueLyser II; TissueRuptor, or mortar and pestle followed by QIAshredder or needle and syringe
	≤30 mg	–	600 µl	

* Use 600 µl Buffer RLT for tissues stabilized in RNA*later*, or for difficult-to-lyse tissues.

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