July 2019

Quick-Start Protocol

miRNeasy Tissue/Cells Advanced Mini Kit

The miRNeasy Tissue/Cells Advanced Mini Kit (cat. no. 217604) is shipped at ambient temperature. All components can be stored dry at room temperature (15–25°C) and are stable for at least 9 months under these conditions if not otherwise stated on the label. This protocol is for purification of total RNA, including small RNAs from animal cells and tissues.

Further information

- *miRNeasy Tissue/Cells Advanced Mini Kit Handbook*: **www.qiagen.com/HB-2672**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Buffer RWT, AL, and RLT contain guanidine salt and are therefore not compatible with disinfecting reagents containing bleach.
- If necessary, redissolve any precipitate in Buffer RLT or Buffer RWT by warming.
- Equilibrate buffers to room temperature.
- All steps should be performed at room temperature. Work quickly.
- Add ethanol (96–100%) to Buffer RWT and Buffer RPE concentrates before use (see bottle label for volume).
- The RNA Spike-in Kit, for RT (cat no. 339390), may be purchased separately. For instructions on preparing a working solution, please refer to the handbook.

- 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.

1. **Cells**: Harvest a maximum of 1 x 10^7 cells either as a cell pellet or lysed directly in the vessel. Add the appropriate volume of Buffer RLT (see Table 1). Vortex for 30 s or homogenize.

   Tissues: Disrupt the tissue (≤30 mg) and homogenize the lysate in the appropriate volume of Buffer RLT (see Table 1).

Table 1. Volumes of buffer RLT for sample disruption and homogenization sample

	Amount	Dish	Buffer RLT	Buffer AL	Disruption and homogenization
Pelleted cells	<5 x 10^6	<6 cm	260 µl	80 µl	Add Buffer RLT, vortex (≤1 x 10^5 cells); or use QIAshredder, TissueRuptor®, or needle and syringe
	≥5 x 10^6	6–10 cm	450 µl	140 µl	
Tissues*	<20 mg	–	260 µl	80 µl	TissueLyser LT, TissueLyser II, TissueRuptor, or mortar and pestle followed by QIAshredder or needle and syringe
	20–30 mg	–	450 µl	140 µl	

* The amount indicated is applicable for fresh or frozen tissue only. For stabilized tissue, use only half of the amount indicated.

2. Add the appropriate volume of Buffer AL (see Table 1) and mix thoroughly. Incubate at room temperature for 3 min. Transfer the homogenized lysate to a gDNA Eliminator spin column placed in a 2 ml collection tube (supplied).

3. Centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm). Discard the column and save the flow-through.

   Optional: Step 4 and 5 do not need to be carried out when working with cell samples.

4. Transfer the flow-through to a new 2 ml reaction vessel (not provided). Add 20 µl Buffer RPP. Close the tube cap and mix vigorously by vortexing for >20 s. Incubate at room temperature for 3 min.

5. Centrifuge at 12,000 x *g* for 3 min at room temperature to pellet the precipitate.

   Note: Supernatant should be clear and colorless. Transfer supernatant (~300 µl) to a new 2 ml reaction tube.

6. Add 1 volume isopropanol and mix well by pipetting. Transfer entire sample to an RNeasy® Mini column. Close the lid and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.

7. Repeat step 6 using the remainder of the sample.

8. Pipet 700 µl Buffer RWT to the RNeasy Mini spin column. Close the lid and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.

9. Pipet 500 µl Buffer RPE onto the RNeasy Mini spin column. Close the lid and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.

10. Add 500 µl of 80% ethanol to the RNeasy Mini spin column. Close the lid and centrifuge for 2 min at ≥8000 x *g*. Discard the flow-through and the collection tube.

11. Place the RNeasy Mini spin column in a new 2 ml collection tube (supplied). Close the lid of the spin column and centrifuge at full speed for 1 min to dry the membrane. Discard the flow-through and the collection tube.

12. Place the RNeasy Mini spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the center of the spin column membrane and incubate for 1 min. Close the lid and centrifuge for 1 min at full speed to elute the RNA.

Document Revision History

Date	Changes
07/2019	Initial release

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.