Quick-Start Protocol March 2016

RNeasy® Maxi Kit

The RNeasy Maxi Kit (cat. no. 75162) can be stored at room temperature (15–25°C) for at least 9 months if not otherwise stated on label.

Further information

- *RNeasy Midi/Maxi Handbook*: **www.qiagen.com/HB-0436**
- 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 from 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 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 tissue stabilized in RNA*later*® from the reagent using forceps.

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

1. **Cells**: Harvest a maximum of 5 x 10^8 cells. Add the appropriate volume of Buffer RLT and homogenize the lysate (see Table 1).

   Tissues: Disrupt and homogenize the tissue (≤1000 mg) in the appropriate volume of Buffer RLT (see Table 1). Centrifuge the lysate for 10 min at 3000–5000 x *g*. 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 shaking vigorously. Do not centrifuge. Proceed immediately to step 3.
3. Transfer the sample (maximum 15 ml) to an RNeasy Maxi column placed in a 50 ml centrifuge tube (supplied). Close the tube, and centrifuge for 5 min at 3000–5000 x *g*. Discard the flow-through.

Optional DNase digest: Follow steps in "Optional on-column DNase digestion with the RNase-Free DNase Set" in Appendix F of the *RNeasy Midi/Maxi Handbook*.

4. Add 15 ml Buffer RW1 to the RNeasy spin column. Close the lid, and centrifuge for 5 min at 3000–5000 x *g*. Discard the flow-through.
5. Add 10 ml Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 2 min at 3000–5000 x *g*. Discard the flow-through.
6. Add 10 ml Buffer RPE to the RNeasy spin column. Close the lid. Centrifuge for 10 min at 3000–5000 x *g* to dry the RNeasy silica membrane.
7. To elute, transfer the RNeasy column to a new 50 ml collection tube (supplied). Add the appropriate volume of RNase-free water (see Table 1) directly to the spin column membrane. Close the tube gently. Let it stand for 1 min, then centrifuge for 3 min at 3000–5000 x *g*.
8. Repeat step 7 as described with a second volume of RNase-free water.

Table 1. Volumes for sample homogenization and elution of RNA

Sample	Amount	Buffer RLT (ml)	Disruption and homogenization	Expected RNA yield and elution volume
Animal cells	5 x 10^7–1.5 x 10^8	7.5	Add Buffer RLT, use TissueRuptor®; or needle and syringe	≤150 µg in 150 µl
	1.5 x 10^8–2.5 x 10^8	7.5 or 15*		150 µg–1 mg in 250 µl
	2.5 x 10^8–5 x 10^8	15		
Animal tissues	150–300 mg	7.5	TissueLyser II; TissueRuptor; or mortar and pestle followed by needle and syringe	≤1 mg in 0.8 ml
	300–500 mg	7.5 or 15†		
	500–1000 mg	15		1–6 mg in 1.2 ml

* If expected RNA yield is higher than 2 mg per 10^8 cells. † If using difficult-to-lyse tissue samples >300 mg.

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