Quick-Start Protocol March 2016

# RNeasy® Midi Kit

The RNeasy Midi Kit (cat. no. 75142) can be stored for at least 9 months at room temperature (15–25°C) 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 RNA*later*® stabilized tissue from the reagent using forceps.
- For RNeasy Protect Midi Kit (cat. no. 75154), please start with the *Quick-StartProtocol RNA*later *RNA Stabilization Reagent, RNAlater TissueProtect Tubes, and RNeasy Protect Kits*.

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

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

   **Tissues**: Disrupt and homogenize the tissue (≤250 mg) in the appropriate volume of Buffer RLT (see Table 1). Centrifuge the lysate for 10 min at 3000–5000 x *g*. 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 4 ml) to an RNeasy Midi column placed in a 15 ml centrifuge tube (supplied). Close the tube gently, and centrifuge for 5 min at 3000–5000 x *g*. Discard the flow-through.
4. Add 4 ml Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 5 min at 3000–5000 x *g*. Discard the flow-through.

   **Optional**: For on-column DNase digestion, follow steps in Appendix F of the *RNeasy Midi/Maxi Handbook*.
5. Add 2.5 ml Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at 3000–5000 x *g*. Discard the flow-through.
6. Add 2.5 ml Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 5 min at 3000–5000 x *g* to dry the RNeasy silica membrane.
7. To elute, transfer the RNeasy column to a new 15 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 $10^6$–3 x $10^7$ | 2 | Add Buffer RLT, use QIAshredder, TissueRuptor®; or needle and syringe | ≤150 µg in 150 µl |
| | 3 x $10^7$–5 x $10^7$ | 2 or 4* | | 150 µg–1 mg in 250 µl |
| | 5 x $10^7$–1 x $10^8$ | 4 | | |
| Animal tissues | 20–75 mg | 2 | TissueLyser II; TissueRuptor; or mortar and pestle followed by QIAshredder or needle and syringe | ≤150 µg in 150 µl |
| | 75–130 mg | 2 or 4† | | 150 µg–1 mg in 250 µl |
| | 130–250 mg | 4 | | |

* If expected RNA yield is higher than 150 µg per $10^7$ cells.

† If using difficult-to-lyse tissue samples >75 mg.

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