Quick-Start Protocol March 2016

# RNeasy® Plus Mini Kit

The RNeasy Plus Mini Kit (cat. nos. 74134 and 74136) can be stored at room temperature (15–25°C) for at least 9 months if not otherwise stated on label.

## Further information

- *RNeasy Plus Mini Handbook*: **www.qiagen.com/HB-0405**
- 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 Plus before use. Buffer RLT Plus 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.
- Foaming can be reduced by adding Reagent DX (cat. no. 19088) at a final concentration of 0.5% (v/v) before disruption and homogenization.*

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

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 Plus (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 Plus (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. 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. Add 1 volume (usually 350 µl or 600 µl) of 70% ethanol to the flow-through, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 4.
4. Transfer up to 700 µl of the sample, including any precipitate, to an RNeasy 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.
5. Add 700 µl Buffer RW1 to the RNeasy Mini spin column (in a 2 ml collection tube). 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 15 s at ≥8000 x *g*. Discard the flow-through.
7. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm).

   **Optional**: Place the RNeasy spin column in a new 2 ml collection tube (supplied). Centrifuge at full speed for 1 min to further dry the membrane.
8. 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.

   **Optional**: Repeat elution with another volume of water or with RNA eluate.

**Table 1. Volumes of Buffer RLT Plus for sample disruption and homogenization**

| Sample | Amount | Dish | Buffer RLT Plus | Disruption and homogenization |
|---|---|---|---|---|
| Pelleted cells | <5 x $10^6$ | <6 cm | 350 µl | Add Buffer RLT Plus, 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 |
| | 20–30 mg | – | 600 µl | |

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

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