Quick-Start Protocol October 2020

# RNeasy® Plus Universal Kits

The RNeasy Plus Universal Mini Kit (cat. no. 73404) and the RNeasy Plus Universal Midi Kit (cat. no. 73442) can be stored at room temperature (15–25°C) for at least 9 months, if not otherwise stated on label. QIAzol® Lysis Reagent can be stored at room temperature or at 2–8°C.

## Further information

- *RNeasy Plus Universal Kits Handbook*: **www.qiagen.com/HB-0391**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

## Notes before starting

- This protocol is for the purification of total RNA. For purifying total RNA containing miRNA using the RNeasy Plus Universal Kits, refer to the *RNeasy Plus Universal Kits Handbook*.
- QIAzol Lysis Reagent and Buffer RWT contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See the "Safety Information" section in the *RNeasy Plus Universal Kits Handbook*.
- Except for phase separation (step 6), all protocol and centrifugation steps should be performed at room temperature (15–25°C). During the procedure, work quickly.
- Add 2 volumes of ethanol (96–100%) to Buffer RWT for a working solution.
- Add 4 volumes of ethanol (96–100%) to Buffer RPE for a working solution.
- **Symbols**: ● RNeasy Plus Universal Mini Kit; ▲ RNeasy Plus Universal Midi Kit

1. Disrupt and homogenize ● ≤50 mg tissue (or ≤100 mg brain or adipose tissue) or ▲ ≤250 mg tissue (or ≤500 mg brain or adipose tissue) in a suitably sized vessel containing ● 900 µl or ▲ 5 ml QIAzol Lysis Reagent using the TissueRuptor®, TissueLyser LT or TissueLyser II.
2. Incubate the homogenate at room temperature (15–25°C) for 5 min.
3. Add ● 100 µl or ▲ 500 µl gDNA Eliminator Solution. Securely cap the tube containing the homogenate and shake it vigorously for 15 s.
4. Add ● 180 µl or ▲ 1 ml chloroform and shake vigorously for 15 s.
5. Incubate sample at room temperature for 2–3 min.
6. Centrifuge at ● 12,000 x *g* or ▲ 5,000 x *g* for 15 min at 4°C.
7. Transfer the upper aqueous phase to a new tube. Be careful to avoid the interphase. Add 1 volume (usually ● 600 µl or ▲ 3 ml) of 70% ethanol and vortex. Do not centrifuge. Proceed immediately to step 8.

8. Transfer up to ● 700 µl of the sample to an RNeasy Mini spin column in a 2 ml collection tube (supplied) or ▲ 4 ml of the sample to an RNeasy Midi Spin column placed in a 15 ml collection tube (supplied). Close the lid, centrifuge at room temperature for ● 15 s at ≥8000 x *g* or ▲ 5 min at 3000–5000 x *g*, and discard flow-through.
9. Using the same collection tube, repeat step 9 using the remainder of the sample. Discard the flow-through.
10. Add ● 700 µl or ▲ 4 ml of Buffer RWT to the RNeasy Spin column. Close the lid, centrifuge for ● 15 s at ≥8000 x *g* or ▲ 5 min at 3000–5000 x *g*, and discard flow-through.
11. Add ● 500 µl or ▲ 2.5 ml Buffer RPE to the RNeasy Spin column. Close the lid, centrifuge for ● 15 s at ≥8000 x *g* or ▲ 2 min at 3000–5000 x *g*, and discard flow-through.
12. Add ● 500 µl or ▲ 2.5 ml Buffer RPE to the RNeasy Spin column. Close the lid, centrifuge for ● 15 s at ≥8000 x *g* or ▲ 5 min at 3000-5000 x *g*, and discard flow-through.
    **Optional**: To further dry the membrane, place the ● RNeasy Mini Spin column in new 2 ml tube, close the lid, and centrifuge at full speed for 1 min.
13. Place RNeasy Spin column in a new ● 1.5 ml or ▲ 15 ml tube. Add ● 30–50 µl or ▲ 150–250µl RNase-free water, close the lid and centrifuge for ● 1 min at ≥8000 x *g* or ▲ 3 min at 3000–5000 x *g*.
    **Optional for RNeasy Plus Universal Mini users**: Repeat step 13 using another volume of RNase-free water or using the eluate from step 13 (if high RNA concentration is required). Reuse the collection tube from step 13.

## Revision History

| Date | Changes |
|---|---|
| 01/2020 | Initial release |
| 10/2020 | Typo correction. |

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