Quick-Start Protocol March 2016

RNeasy® Plus Micro Kit

The RNeasy Plus Micro Kit (cat. no. 74034) is shipped at ambient temperature. Store the RNeasy MinElute® spin columns immediately upon receipt at 2–8°C. Store the remaining components of the kit dry at room temperature (15–25°C). All kit components are stable for at least 9 months under these conditions if not otherwise stated on label.

Further information

- *RNeasy Plus Micro Handbook*: **www.qiagen.com/HB-1950**
- 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.
- When processing <500 cells or <2 µg tissue, carrier RNA may be added to the lysate before homogenization (see the *RNeasy Plus Micro Handbook* for further information).
- 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 the kit handbook; handbook to be updated.

1. **Cells**: Harvest a maximum of 5 x 10^5 cells, as a cell pellet, or by direct lysis in the cell-culture dish (up to 10 cm diameter). Add 350 µl Buffer RLT Plus. Homogenize the lysate.

   Tissues: Do not use more than 5 mg tissue. Add 350 µl of Buffer RLT Plus. Disrupt and homogenize the tissue using the TissueRuptor® or TissueLyser instruments. Centrifuge the lysate for 3 min at maximum speed. Remove the supernatant by pipetting and use it in step 2.

Microdissected cryosections: Collect the sample directly into an appropriate volume of Buffer RLT Plus. (The volume depends on the collection vessel used for microdissection, but should not exceed 65 µl [Leica® instruments] or 300 µl [other instruments].) Adjust the volume to 350 µl with Buffer RLT Plus. Vortex for 30 s.

2. Transfer the lysate to a gDNA Eliminator spin column placed in a 2 ml collection tube (supplied). Centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm). Discard the column, and save the flow-through.
3. Add 1 volume (usually 350 µl) of 70% ethanol to the flow-through, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 4.
4. Transfer the sample, including any precipitate that may have formed, to an RNeasy MinElute 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 MinElute spin column. 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 MinElute spin column. Close the lid, and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.
7. Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid, and centrifuge for 2 min at ≥8000 x *g* to wash the spin column membrane. Discard the collection tube with the flow-through.
8. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min to dry the membrane. Discard the collection tube with the flow-through.
9. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.

Scan QR code for handbook.