Quick-Start Protocol December 2017

RNeasy® UCP Micro Kit

The RNeasy UCP Micro Kit (cat. no. 73934) is shipped at ambient temperature. Store the RNeasy UCP MinElute® spin columns and the RNase-Free DNase Set immediately upon receipt at 2–8°C. Store the remaining components dry at room temperature (15–25°C). All kit components are stable for at least 9 months under these conditions.

Further information

- *RNeasy UCP Micro Kit Handbook*: **www.qiagen.com/HB-2302**
- 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 RULT before use. Buffer RULT containing DTT or β-ME can be stored at room temperature for up to 1 month.
- Buffers RUWT and RUPE are supplied as concentrates. Before using for the first time, add the required volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.
- To prepare DNase I stock solution, dissolve the lyophilized DNase I in 550 µl RNase-free water. Mix gently by inverting the vial. Do not vortex.
- Store DNase I as single-use aliquots at –15 to –30 °C for up to 9 months or store at 2–8°C for up to 6 weeks. Do not refreeze after thawing.

Procedure

1. **Cells**: Harvest a maximum of 5 x 10^5 cells as a cell pellet or by direct lysis in the vessel. Add 350 µl Buffer RULT and homogenize.

   Tissues: Disrupt and homogenize ≤5 mg tissue in 350 µl Buffer RULT using the TissueRuptor® or TissueLyser instrument. Centrifuge the lysate for 3 min at maximum speed. Carefully remove the supernatant by pipetting, and use for step 2.
2. Add 1 volume of 70% ethanol to the lysate and mix well by pipetting. Do not centrifuge. Proceed immediately to step 3.
3. Transfer the sample, with any precipitate, to an RNeasy UCP MinElute spin column in a 2 ml collection tube (supplied). Close the lid and centrifuge for 15 s at ≥8000 *x g*. Discard the flow-through.
4. Add 350 µl Buffer RUWT to the RNeasy UCP MinElute spin column. Close the lid. Centrifuge for 15 s at ≥8000 *x g*. Discard the flow-through.
5. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by inverting the tube. Add the DNase I incubation mix (80 µl) directly to the RNeasy UCP MinElute spin-column membrane. Place on the benchtop (15 to 25 °C) for 15 min. Add 350 µl Buffer RUWT to the RNeasy UCP MinElute spin column. Close the lid and centrifuge for 15 s at ≥8000 *x g*. Discard the collection tube.
6. Place the RNeasy UCP MinElute spin column in a new 2 ml collection tube (supplied). Add 500 µl Buffer RUPE to the 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 UCP MinElute spin column. Close the lid and centrifuge for 2 min at ≥8000 *x g*. Discard the collection tube.
8. Place the RNeasy UCP 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 flow-through and collection tube.
9. Place the RNeasy UCP 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.

Revision history

Document	Date	Description of changes
RNeasy UCP Micro Kit Quick-Start Protocol	September 2016	Initial release
RNeasy UCP Micro Kit Quick-Start Protocol	December 2017	Updated details for preparing working solutions of Buffers RUWT and RUPE