January 2020

Quick-Start Protocol

AllPrep® DNA/RNA Micro Kit

The AllPrep DNA/RNA Micro Kit (cat. no. 80284) should be stored at room temperature (15–30°C) and is stable for at least 9 months under these conditions, if not otherwise stated on label. RNeasy MinElute spin columns should be stored at 2–8°C.

Further information

- *AllPrep DNA/RNA Micro Handbook*: **www.qiagen.com/HB-1951**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for the simultaneous purification of DNA and total RNA from animal cells or human cells. For purifying DNA and total RNA from animal or human tissues or from microdissected cryosections, refer to the *AllPrep DNA/RNA Micro Handbook*.
- Buffer RLT Plus, Buffer RW1, and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See the "Safety Information" section in the *AllPrep DNA/RNA Micro Handbook*.
- All procedures need to be performed at room temperature (15–25°C). Work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.
- β-mercaptoethanol (β-ME) must be added to Buffer RLT if working with cell lines rich in RNases. Add 10 µl ß-ME per 1 ml buffer RLT. Buffer RLT containing ß-ME can be stored at room temperature for up to 1 month.

- Buffer RPE, Buffer AW1, and Buffer AW2 are each supplied as concentrate. Before using them for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Buffer RLT may form a precipitate during storage. If necessary, redissolve by warming and then place at room temperature.
- Preheat Buffer EB to 70°C to ensure optimal DNA elution.

1. Harvest a maximum of 5 x 10^5 cells, either as a cell pellet or by direct lysis in the cell-culture dish (up to 10 cm diameter).
2. Add 350 µl Buffer RLT Plus. If the number of pelleted cells is below 1 x 10^5, add 75 µl Buffer RLT Plus. This may be necessary for multiwell plates and cell-culture dishes. Pipet up and down to lyse the cells.
3. Homogenize the lysate using one of 3 possible methods below:

   Note: If only 75 µl Buffer RLT Plus was used in step 2, transfer the lysate to a new 1.5 ml microcentrifuge tube and adjust the volume to 350 µl with Buffer RLT Plus. Vortex for 1 min to homogenize and proceed to step 4.

   Note: If processing <500 cells, 20 ng carrier RNA (5 µl of a 4 ng/µl solution) may be added to the lysate before homogenization.

   3a. Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube. Centrifuge for 2 min at full speed. Proceed to step 4.

   3b. Place the tip of the TissueRuptor® disposable probe into the lysate, and then operate the TissueRuptor at full speed until the lysate is homogenous (usually 30 s). Proceed to step 4.

   3c. Pass the lysate at least 5 times through a blunt 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe. Proceed to step 4.
4. Transfer the homogenized lysate to an AllPrep DNA spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and then centrifuge for 30 s at >8000 x *g* (>10,000 rpm).

5. Place the AllPrep DNA spin column in a new 2 ml collection tube (supplied) and store at room temperature for later DNA purification in steps 13 to 16. Use the flow-through for RNA purification in steps 6 to 12.

   Note: Do not store the AllPrep DNA spin column at room temperature or at 4°C for long periods. Do not freeze the column.

Total RNA purification

6. Add 1 volume (usually 350 µl) of 70% ethanol to the flow-through from step 5, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 7.
7. 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 gently, and then centrifuge for 15 s at >8000 x *g* (>10,000 rpm). Discard the flow-through. Reuse the collection tube in step 8.
8. Add 700 µl Buffer RW1 to the RNeasy MinElute spin column. Close the lid gently, and then centrifuge for 15 s at >8000 x *g* (>10,000 rpm) to wash the spin column membrane. Discard the flow-through. Reuse the collection tube in step 9.
9. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and then centrifuge for 15 s at >8000 x *g* (>10,000 rpm) to wash the spin column membrane.

   Discard the flow-through. Reuse the collection tube in step 10.
10. Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the collection tube with the flow-through.
11. 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. Discard the collection tube with the flow-through.
12. 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.

Genomic DNA purification

13. Add 500 µl Buffer AW1 to the AllPrep DNA spin column from step 5. Close the lid gently, and centrifuge for 15 s at >8000 x *g* (10,000 rpm) to wash the spin column membrane. Discard the flow-through. Reuse the spin column in step 22.
14. Add 500 µl Buffer AW2 to the AllPrep DNA spin column. Close the lid gently, and centrifuge for 2 min at full speed to wash the spin column membrane.
15. Place the AllPrep DNA spin column in a new 1.5 ml collection tube (supplied). Add 50 µl Buffer EB (preheated to 70°C) directly to the spin column membrane and close the lid. Incubate at room temperature (15–25°C) for 2 min, and centrifuge for 1 min at ≥8000 x *g* (10,000 rpm) to elute the DNA.
16. Repeat step 15 to further elute DNA.

Revision History

Date	Changes
01/2020	Initial release

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.