November 2019

Quick-Start Protocol

AllPrep® DNA/RNA/Protein Mini Kit

The AllPrep DNA/RNA/Protein Mini Kit (cat. no. 80004) should be stored at room temperature (15–30°C) and is stable for at least 6 months under these conditions.

Further information

- *AllPrep DNA/RNA/Protein Mini Handbook*: **www.qiagen.com/HB-0447**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for the simultaneous purification of DNA, total RNA and protein from animal cells or human cells. For purifying DNA, total RNA and protein from animal or human tissues, refer to the *AllPrep DNA/RNA/Protein Mini Handbook*.
- Buffer RLT, 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/Protein Mini 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 before use. 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.
- Dithiothreitol (DTT) must be added to Buffer ALO before use. Add 8 mg DTT per 1 ml Buffer ALO.
- 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 1 x 10^7 cells, either as a cell pellet or by direct lysis in the cell-culture dish (up to 10 cm diameter).
2. Add 350 µl if the number of pelleted cells is below 5 x 10^6. Add 600 µl if 5 x 10^6 to 1 x 10^7 cells are used.
3. Homogenize the lysate using one of 3 possible methods:
   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 21–24. Use the flow-through for RNA purification in steps 6–13.

   Note: DNA purification is best done as soon as possible, following the steps of this protocol. If, for some reason, delay is unavoidable, store the spin column at 4°C instead.

Total RNA purification

6. Add 96–100% ethanol to the flow-through (see step 4). Use 250 µl ethanol if 350 µl Buffer RLT was used. Use 400 µl ethanol if 600 µl Buffer RLT was used. Mix well by pipetting. Do not centrifuge. Proceed immediately to the next step.

7. Transfer up to 700 µl of the sample – including any precipitate that may have formed – to an RNeasy® 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).

   Transfer the flow-through to a 2 ml tube (supplied) for protein purification in steps 14–20. Reuse the collection tube in step 8.

8. Add 700 µl Buffer RW1 to the RNeasy 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 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 Buffer RPE to the RNeasy spin column. Close the lid gently, and then centrifuge for 2 min at >8000 x *g* (>10,000 rpm) to wash the spin column membrane.

11. **Optional**: To avoid carryover of Buffer RPE, place the RNeasy spin column in a new 2 ml collection tube (supplied) and discard the old collection tube with the flow-through. Centrifuge at full speed for 1 min.

12. 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 gently, and then centrifuge for 1 min at >8000 x *g* (>10,000 rpm) to elute the RNA.

13. If the expected RNA yield is >30 µg, repeat step 12 using another 30–50 µl of RNase-free water or, if high RNA concentration is required, using the eluate from step 12. Reuse the collection tube from step 12.

Total protein precipitation

14. Add 1 volume (usually 600 or 1000 µl) of Buffer APP to the flow-through from step 7. Mix vigorously and incubate at room temperature for 10 min to precipitate protein.

15. Centrifuge at full speed for 10 min, and then carefully decant the supernatant.

16. Add 500 µl of 70% ethanol to the protein pellet. Centrifuge at full speed for 1 min, and then remove the supernatant by using a pipet or by decanting as much liquid as possible.

17. Dry the protein pellet for 5–10 min at room temperature.

18. Add up to 100 µl Buffer ALO and mix vigorously to dissolve the protein pellet.
19. Incubate for 5 min at 95°C to completely dissolve and denature the protein, and then cool the sample to room temperature.
20. Centrifuge for 1 min at full speed to pellet any residual insoluble material. Use the supernatant in downstream applications such as SDS-PAGE and western blotting.

Genomic DNA purification

21. Add 500 µl Buffer AW1 to the AllPrep DNA spin column from step 5. 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 spin column in step 22.
22. Add 500 µl Buffer AW2 to the AllPrep DNA spin column. Close the lid gently, and then centrifuge for 2 min at full speed to wash the spin column membrane.
23. Place the AllPrep DNA spin column in a new 1.5 ml collection tube (supplied). Add 100 µl Buffer EB (preheated to 70°C) directly to the spin column membrane and close the lid. Incubate at room temperature for 2 min, and then centrifuge for 1 min at >8000 x *g* (10,000 rpm) to elute the DNA.
24. Repeat step 23 to elute further DNA.

Document Revision History

Date	Changes
11/2019	In "Notes Before Starting", the quantity of Buffer ALO was changed from 11 ml to 1 ml.
02/2019	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.