February 2021

Quick-Start Protocol

AllPrep® 96 DNA/RNA Kit

All reagents and components of the AllPrep 96 DNA/RNA Kit should be stored at room temperature (15–25°C) and are stable for at least 9 months under these conditions.

Further information

- *AllPrep 96 DNA/RNA Handbook*: **www.qiagen.com/HB-1952**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for simultaneous purification of DNA and RNA from 1 x 10^6 cells using the vacuum/spin technology.
- All vacuum steps are performed on the QIAvac 96 vacuum manifold.
- All centrifugation steps are performed in the Centrifuge 4-16KS with the Plate Rotor 2 x 96.
- Use of a multichannel pipet is recommended. Pour buffers and RNase-free water into reagent reservoirs for multichannel pipets. Use reservoirs from a freshly opened package or clean them as described for S-Blocks.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly. Perform all centrifugation steps at 20–25°C. Ensure that the centrifuge does not cool below 20°C.
- If purifying RNA from cell lines rich in RNases, we recommend adding β-mercaptoethanol (β-ME) to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature (15–25°C) for up to 1 month. Alternatively, add 20 µl of 2 M dithiothreitol (DTT) per 1 ml Buffer RLT. The stock solution of 2 M DTT in water should be prepared fresh or kept frozen in single-use aliquots. Buffer RLT containing DTT can be stored at room temperature for up to 1 month.
- Buffer RLT, Buffer RW1, and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach.

- Buffer RPE, Buffer AW1, and Buffer AW2 are each supplied as a concentrate. Before using 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 upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Preheat Buffer EB to 70°C to ensure optimal DNA elution.

Using vacuum/spin technology

Sample lysis and homogenization

1. Harvest cells (up to 1 x 10^6 cells) according to step 1a or 1b.
   1a. Cells grown in a monolayer: Completely remove the cell-culture medium by pipetting, and add 300 µl Buffer RLT to each well. Transfer the lysates to a rack of collection microtubes, and seal the tubes with collection microtube caps .
   1b. Cells grown in suspension: Transfer up to 1 x 10^6 cells from each sample to a rack of collection microtubes. Pellet the cells by centrifuging for 5 min at 300 x *g*. Completely remove all supernatant by pipetting, and add 300 µl Buffer RLT to each tube. Seal the tubes with collection microtube caps.
2. Homogenize the lysates by vortexing the rack of collection microtubes at full speed for at least 30 s.
3. Place an AllPrep 96 DNA plate on top of a new S-Block. Mark the plate for later identification.
4. Transfer the lysates from step 2 to the wells of the AllPrep 96 DNA plate.
5. Seal the AllPrep 96 DNA plate with an AirPore Tape Sheet. Place the S-Block and AllPrep 96 DNA plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (approx. 5600 x *g*) for 4 min at 20–25°C.
6. Place the AllPrep 96 DNA plate on top of another S-Block (either new or reused), and store at room temperature (15–25°C) or at 4°C for later DNA purification in steps 17–22. Keep the S-Block containing the flow-through for RNA purification in steps 7–16.

Total RNA purification

7. Assemble the QIAvac 96 vacuum manifold: First, place the waste tray inside the QIAvac base, then place the QIAvac 96 top plate squarely over the QIAvac base. Place an RNeasy® 96 plate in the QIAvac 96 top plate, making sure that the plate is seated tightly. Attach the vacuum manifold to a vacuum source. Keep the vacuum switched off.

8. Add 1 volume (300 µl) of 70% ethanol to each well of the S-Block containing the flow-through from step 6. Mix well by pipetting up and down 3 times.
9. Transfer the samples (600 µl) to the wells of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the samples have completely passed through the membranes (15–60 s). Switch off the vacuum, and ventilate the manifold.
10. Add 800 µl Buffer RW1 to each well of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the buffer has completely passed through the membranes (10–30 s). Switch off the vacuum, and ventilate the manifold.
11. Lift the QIAvac 96 top plate carrying the RNeasy 96 plate from the QIAvac base, and empty the waste tray. Reassemble the QIAvac 96 vacuum manifold.
12. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and switch on the vacuum. Apply the vacuum until the buffer has completely passed through the membranes (10–30 s). Switch off the vacuum, and ventilate the manifold.
13. Place the RNeasy 96 plate on top of an S-Block (either new or reused). Mark the plate for later identification.
14. Add 800 µl Buffer RPE to each well of the RNeasy 96 plate, and seal the plate with an AirPore Tape Sheet. Place the S-Block and RNeasy 96 plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (approx. 5600 x *g*) for 10 min at 20–25°C to dry the membranes.
15. Remove the AirPore Tape Sheet. Place the RNeasy 96 plate on top of a rack of Elution Microtubes CL. Add 45–70 µl RNase-free water to each well, and seal the plate with a new AirPore Tape Sheet. Incubate for 1 min at room temperature (15–25°C). Then centrifuge at 6000 rpm (approx. 5600 x *g*) for 4 min at 20–25°C to elute the RNA.
16. Remove the AirPore Tape Sheet. Repeat step 15 with a second volume of 45–70 µl RNase-free water.

Genomic DNA purification

17. Assemble the QIAvac 96 vacuum manifold: First, place the waste tray inside the QIAvac base, then place the QIAvac 96 top plate squarely over the QIAvac base. Place the AllPrep 96 DNA plate (from step 6) in the QIAvac 96 top plate, making sure that the plate is seated tightly. Attach the vacuum manifold to a vacuum source. Keep the vacuum switched off.
18. Add 800 µl Buffer AW1 to each well of the AllPrep 96 DNA plate, and switch on the vacuum. Apply the vacuum until the buffer has completely passed through the membranes (10–30 s). Switch off the vacuum, and ventilate the manifold.

19. Place the AllPrep 96 DNA plate on top of an S-Block (either new or reused).
20. Add 800 µl Buffer AW2 to each well of the AllPrep 96 DNA plate, and seal the plate with an AirPore Tape Sheet. Place the S-Block and AllPrep 96 DNA plate into the metal holder, and place the whole assembly into the rotor bucket. Centrifuge at 6000 rpm (approx. 5600 x *g*) for 10 min at 20–25°C to dry the membranes.
21. Remove the AirPore Tape Sheet. Place the AllPrep 96 DNA plate on top of a rack of Elution Microtubes CL. Add 50–100 µl Buffer EB (prewarmed to 70°C) to each well, and seal the plate with a new AirPore Tape Sheet. Incubate for 5 min at room temperature (15–25°C). Then centrifuge at 6000 rpm (approx. 5600 x *g*) for 4 min at 20–25°C to elute the DNA.
22. Remove the AirPore Tape Sheet. Repeat step 21 with a second volume of 50–100 µl Buffer EB.

Document Revision History

Date	Changes
02/2021	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.