Quick-Start Protocol July 2019

QIAamp® ccfDNA/RNA Kit

The QIAamp ccfDNA/RNA Kit (cat. no. 55184) is shipped at ambient temperature. Upon arrival, store the RNeasy® MinElute® spin columns at 2–8°C. Store the remaining components dry at room temperature (15–25°C). This protocol is for purification of total circulating cell-free DNA and RNA, including RNA inside of exosomes and other extracellular vesicles (EVs), from 1–4 ml of serum or EDTA plasma.

Further information

- *QIAamp ccfDNA/RNA Kit Handbook*: **www.qiagen.com/HB-2389**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- If necessary, re-dissolve any precipitate in Buffer RPL by warming and then equilibrate back to room temperature.
- Buffer RWT and RPL contain guanidine salt and are therefore not compatible with disinfecting reagents containing bleach.
- Equilibrate buffers to room temperature. Keep isopropanol on ice.
- Except isopropanol addition (Step 6), all steps should be performed at room temperature. Work quickly.
- Add ethanol (96–100%) to Buffer RWT and Buffer RPE concentrates before use (see bottle label for volume).
- Before starting, read the recommendations for preparing serum or plasma in the *QIAamp ccfDNA/RNA Kit Handbook*.

1. Prepare serum or plasma or thaw frozen samples.
2. Transfer between 1–4 ml serum or plasma into a 15 ml collection tube.
3. Add 300 µl Buffer RPL for each 1 ml of plasma or serum. Close the tube cap and vortex for 5 s. Leave at room temperature for 3 min.
4. Add 100 µl Buffer RPP for each 1 ml of plasma or serum. Close the tube cap and immediately mix vigorously by vortexing for >20 s. Incubate on ice for 3 min.
5. Centrifuge at 12000 *x g* for 3 min to pellet the precipitate.

   Note: Supernatant should be clear and colorless.
6. Transfer supernatant (about 1 ml per ml of serum or plasma) to a new tube. Keep on ice.
7. Add 1 volume ice-cold isopropanol to each well. Thoroughly mix by vortexing.
8. Pipet up to 4 ml sample, including any precipitate, onto an RNeasy Midi spin column in a 15 ml collection tube. Close the lid and centrifuge at ≥5000 *x g* for 1 min at room temperature in a swinging bucket rotor. Discard the flow-through.
9. Repeat step 8 using the remainder of the sample (if any).

   Note: If any liquid remains, centrifuge again at ≥5000 *x g* for 1 min.
10. Add 4 ml Buffer RWT to the RNeasy Midi spin column. Close the lid, and centrifuge for 1 min at ≥5000 *x g*. Discard the flow-through.
11. Pipet 2.5 ml Buffer RPE onto the RNeasy Midi spin column. Close the lid, and centrifuge for 5 min at ≥5000 *x g*. Discard the flow-through.
12. Place the RNeasy Midi spin column in a new 15 ml collection tube (supplied). Add 200 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, incubate for 1 min, and then centrifuge for 1 min at full speed to elute the DNA/RNA.

Cleanup (automatable on QIAcube® and QIAcube Connect)

1. Add 200 µl Buffer RPL to 200 µl eluate.
2. Add 800 µl ethanol (96–100%) and mix by pipetting or vortexing.
3. Pipet 700 µl sample, including any precipitate, onto an RNeasy MinElute spin column in a 2 ml collection tube. Close the lid and centrifuge at ≥8000 *x g* for 15 s at room temperature. Discard the flow-through.
4. Repeat step 3 using the remainder of the sample.
5. Pipet 500 µl Buffer RPE onto 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 of 80% ethanol to the RNeasy MinElute spin column. Close the lid and centrifuge for 15 s at ≥8000 *x g*. Discard the flow-through and the collection tube.
7. 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 flow-through and the collection tube.
8. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14–20 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, incubate for 1 min, and centrifuge for 1 min at full speed to elute the DNA/RNA.

Document Revision History

Date	Changes
August 2017	Initial release
July 2019	Correction in Step 4 of the cleanup protocol from “step 16” to “step 3”. Change in QR code redirect from the quick-start protocol to the kit handbook.

Scan QR code for handbook.