

## QIAseq cfDNA Extraction Kit

Store the QIAamp Mini columns at 2–8°C upon arrival. Columns are stable for at least one year after delivery. Short-term storage (up to 4 weeks) at room temperature (15–25°C) will not adversely affect performance of the columns. All buffers can be stored at room temperature (15–25°C) for up to one year. This protocol is for the extraction of circulating cell-free DNA (cfDNA) for next-generation sequencing from plasma.

### Further Information

- *QIAseq cfDNA All-in-One Kit Handbook*: [www.qiagen.com/HB-2151](http://www.qiagen.com/HB-2151)
- Safety Data Sheets: [www.qiagen.com/safety](http://www.qiagen.com/safety)
- Technical assistance: [support.qiagen.com](http://support.qiagen.com)

### Notes before starting

- Buffers ACB, ACW1 and ACW2 are buffer concentrates and need to be prepared in advance according to the instructions provided in the handbook (see “Preparation of buffers”).
- Refer to the kit handbook for setup and handling guidelines for the QIAvac 24 Plus vacuum manifold.

### Protocol: Purification of Circulating Nucleic Acids from 5ml Plasma

This protocol is for the purification of circulating DNA from 5 ml of plasma. For other plasma input volumes, see detailed protocols in the Handbook.

### Notes before starting

- Equilibrate samples to room temperature.

- If samples are <5 ml, bring their volumes up to 5 ml with phosphate-buffered saline.
- Set up the QIAvac 24 Plus as described in the kit handbook.
- Pre-heat a water bath or heating block to 60°C for 50 ml centrifuge tubes in step 4.
- Pre-heat a heating block to 56°C for use with 2 ml collection tubes in step 14.
- Equilibrate Buffer AVE at room temperature (for elution in step 15).

## Procedure

1. Pipet 500 µl QIAGEN Proteinase K into a 50 ml centrifuge tube (not provided).
2. Add 5 ml of plasma to the tube.
3. Add 4 ml Buffer ACL. Close the cap and mix by pulse-vortexing for 30 s.

Make sure that a visible vortex forms in the tube. To ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.

**Note:** Do not interrupt the procedure at this time. Proceed immediately to step 4 to start the lysis incubation.

4. Incubate at 60°C for 30 min.
5. Place the tube back on the lab bench and unscrew the cap.
6. Add 9 ml Buffer ACB to the lysate in the tube. Close the cap and mix thoroughly by pulse-vortexing for 15–30 s.
7. Place the lysate-Buffer ACB mixture in the tube for 5 min on ice.
8. Insert the QIAamp Mini column into the VacConnector on the QIAvac 24 Plus. Insert a 20 ml tube extender into the open QIAamp Mini column. Make sure that the tube extender is firmly inserted into the QIAamp Mini column in order to avoid sample leakage.

**Note:** Keep the collection tube for the dry spin in step 13.

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- Carefully transfer the lysate-Buffer ACB mixture from step 7 into the tube extender of the QIAamp Mini column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender.

Please note that large sample lysate volumes (about 20 ml when starting with 5 ml sample) may need up to 15 minutes to pass through the QIAamp Mini membrane when using a vacuum. For fast and convenient release of the vacuum pressure, the Vacuum Regulator should be used (part of the QIAvac Connecting System).

**Note:** To avoid cross-contamination, be careful not to move the tube extenders over neighboring QIAamp Mini Columns.

- Apply 600  $\mu$ l Buffer ACW1 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
  - Apply 750  $\mu$ l Buffer ACW2 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
  - Apply 750  $\mu$ l of ethanol (96–100%) to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of ethanol has been drawn through the spin column, switch off the vacuum pump and release the pressure to 0 mbar.
  - Close the lid of the QIAamp Mini column. Remove it from the vacuum manifold, and discard the VacConnector. Place the QIAamp Mini column in a clean 2 ml collection tube, and centrifuge at full speed (20,000  $\times$  g; 14,000 rpm) for 3 min.
  - Place the QIAamp Mini Column into a new 2 ml collection tube. Open the lid, and incubate the assembly at 56°C for 10 min to completely dry the membrane.
  - Place the QIAamp Mini column in a clean 1.5 ml elution tube (provided) and discard the 2 ml collection tube from step 14. Carefully apply 40–60  $\mu$ l of Buffer AVE to the
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center of the QIAamp Mini membrane. Close the lid and incubate at room temperature for 3 min.

**Important:** Ensure that the elution buffer AVE is equilibrated to room temperature (15–25°C). Dispense the elution buffer onto the center of the membrane for complete elution of bound DNA. The recovered eluate volume will be up to 5 µl less than the elution volume applied to the QIAamp Mini column.

16. Centrifuge in a microcentrifuge at full speed (20,000 x g; 14,000 rpm) for 1 min to elute the nucleic acids.



Scan QR code for handbook.

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

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