Application Note

Quality assessment of cell-free DNA (cfDNA) using the QIAxcel capillary gel electrophoresis system

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Introduction

cfDNA refers to the remaining DNA in biofluids after removing cells and cell debris in healthy and diseased subjects. It can detect biomarkers for cancer research and is widely used in many research fields. As cellular DNA contamination influences the quantification of cfDNA yield, quality assessment of isolated cfDNA is crucial for further downstream applications. Capillary gel electrophoresis offers a reliable method to evaluate cfDNA quality based on expected size and fragmentation pattern and the absence of undesired products in the sample (1-2).

This application note aims to discuss how the QIAxcel Connect capillary gel electrophoresis system is used to evaluate the size, peak pattern, and purity of isolated cfDNA.

Materials and Methods

Sample preparation

Human plasma was generated from EDTA blood collection tubes from either freshly collected blood or blood stored at room temperature (RT) for seven days. Isolation of cfDNA from plasma samples was automated using the EZ1&2 ccfDNA Kit on the EZ2 Connect instrument. The concentration of the cfDNA was measured using the Qubit™ dsDNA HS assay kit (Thermo Fisher Scientific) on the Qubit 3.0 fluorometer (Table 1).

Run parameters

The quality assessment of cfDNA samples was done using the QIAxcel Connect system (3). The QIAxcel DNA High Sensitivity Kit was used with the run method DNA High-Sensitivity_V2.

Alignment Marker QX 15 bp HS (diluted QX RNA Alignment Marker) and Size Marker QX DNA HS 100 bp - 1kb (provided with QIAxcel DNA High Sensitivity Kit) were used for this analysis.

Table 1. Overview of cfDNA samples

	Input plasma volume (mL)	Elution volume (µL)	Concentration (ng/µL)	Condition
Sample 1	10	45	1.7	Fresh
Sample 2a	2	75	0.15	Fresh
Sample 2b	2	75	3.2	Stored for 7 days at RT

Results and Discussion

Resolution and sizing of cfDNA

cfDNA typically has a predominant fragment size range of around 166–170 bp. This corresponds to the length of DNA that can wrap around a nucleosome (147 bp), plus an additional stretch to link two nucleosome cores. Apoptosis can also produce longer cfDNA fragments that correspond to di- (~350 bp), tri- (~565 bp) or poly-nucleosomes (4). Figure 1 shows the electropherogram of isolated cfDNA (1.7 ng/µL) from human plasma. The mononucleosomal (178 bp) and the dinucleosomal peak (408 bp) is detected. No undesired products were detected.

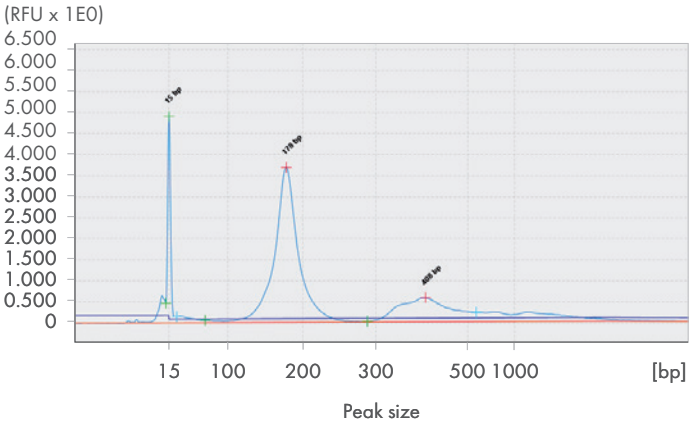

Figure 1. Electropherogram of isolated cfDNA from human plasma.

cfDNA containing genomic DNA (gDNA)

When blood samples are not directly processed, cell death occurs and gDNA is released. DNases present in the blood cause gDNA fragmentation, resulting in various fragment sizes (5). The electropherogram in Figure 2a shows the mononucleosomal peak of cfDNA (172 bp) from human plasma generated from freshly collected blood. No undesired products were detected in the sample. Figure 2b shows the electropherogram from the same sample after storage at RT for seven days before plasma generation and cfDNA isolation. Contamination of the sample with fragmented gDNA is visible based on the various fragments that are detected. An increase in the signal of the mononucleosomal peak (174 bp) is clearly visible. A peak in the size of dinucleosomal DNA (399 bp) is also detected. In addition, gDNA fragments of bigger sizes are also present in the sample.

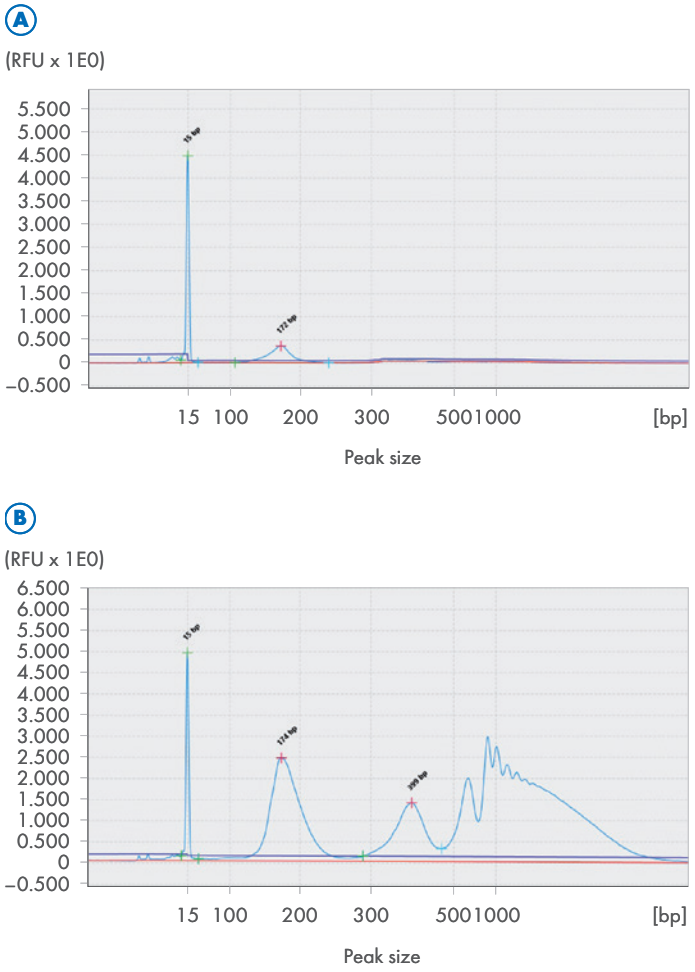

Figure 2. Electropherograms of cfDNA samples with and without gDNA contamination. A The resolution and sizing of cfDNA isolated from freshly collected blood are shown. **B** The same sample was analyzed for resolution and size on the QIAxcel Connect System after storage for seven days at RT before plasma generation and cfDNA isolation.

Conclusion

cfDNA-based testing is a less-invasive approach that can detect biomarkers in cancer and other research fields. In addition to quantification, quality assessment of cfDNA, consisting of sizing, peak pattern and purity testing, is crucial for further downstream applications.

The QIAxcel Connect System can reliably detect low amounts of cfDNA, showing up as a mononucleosomal peak in the electropherogram. As the absence of contamination with other DNA is crucial for further processing of cfDNA samples, capillary gel electrophoresis is a valid and valuable method to examine the purity of a cfDNA sample.

References

1. Chen, Y.L., et al., *Five Technologies for Detecting the EGFR T790M Mutation in the Circulating Cell-Free DNA of Patients With Non-small Cell Lung Cancer: A Comparison*. Front. Oncol., 2019. **19**(631)
2. Udomruk S., et al., *Characterization of Cell-Free DNA Size Distribution in Osteosarcoma Patients*. Clinical Cancer Research, 2023. **29**(11)
3. QIAxcel Connect User Manual. QIAGEN. 2022. Downloaded from qiagen.com/HB-2890
4. Bronkhorst, A.J., et al., *The emerging role of cell-free DNA as a molecular marker for cancer management*. Biomol. Detect. Quantif., 2019. **17**
5. Nagata, S., *Apoptotic DNA Fragmentation*, 2000. **256**(1): p.12-18

Ordering Information

Product	Contents	Cat. no.
QIAxcel Connect	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel Software, and 1-year warranty on parts and labor	9003110
QIAxcel DNA High Sensitivity Kit (1200)	QIAxcel DNA High Sensitivity Cartridge, QIAxcel DNA High Sensitivity Marker Set, Buffers, Mineral Oil, 12-Tube Strips	929012
QX RNA Alignment Marker (1.5 mL)	Single 15 BP fragment for alignment	929510

The QIAxcel Connect is intended for molecular biology applications only. The products mentioned in this guide are not intended for the diagnosis, prevention or treatment of a disease.

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For more information, visit: **qiagen.com/QIAxcel-Connect**