Application Note

Effect of preanalytical factors on analyte quality as shown with the QIAxcel Connect capillary gel electrophoresis system

Applying QIAxcel® Connect capillary gel electrophoresis system for quality control of samples collected and stabilized with PAXgene® products

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Introduction

PAXgene products [1] are specifically developed as integrated and standardized systems for sample collection, stabilization and purification of high-quality nucleic acids from human samples. The collection and stabilization devices contain innovative stabilization additives which enable convenient, room-temperature stabilization of the molecular profile during transport and handling, as sample handling protocols and storage times until processing can vary among collection sites.

Blood and urine are common body fluids that can be easily collected and analysed for biomarkers like circulating cell-free DNA (ccfDNA), cell-free DNA (cfDNA) and genomic DNA (gDNA) making them valuable for less- or non-invasive liquid biopsy applications in disease research, diagnostics and monitoring.

cfDNA is released into body fluids by biological processes such as apoptosis or necrosis and remains in the fluid after the removal of cells and cell debris. cfDNA is only present in low quantities in blood and urine. Enzymatic degradation in body fluids after collection can even further reduce the quantity of cfDNA and affect the analysis. Cellular DNA released from cells after body fluid collection dilutes the cfDNA and further influences the quantification of cfDNA yield. Additionally, sensitivity of an analytical assay is strongly dependent on the yield, integrity and quality of the analytes of interest. The choice of the sample collection and stabilization device and technology used may have an influence on the limit of detection (LOD) of the assay affecting downstream analysis.

Therefore, the preanalytical workflow which delivers these analytes is the key part for the correct and reliable analytical test results and optimization might result in detecting more and higher quality cfDNA in downstream analysis [2]. The PAXgene Blood ccfDNA Tube* and the PAXgene Urine Liquid Biopsy Set† help to minimize the described processes above and thereby lead to the preservation of cfDNA profiles in blood and urine samples respectively.

gDNA can be used in various areas of diagnostics and research like targeted analysis of genetic disorders or whole genome sequencing approaches. However, inconsistent blood draw volumes and different anticoagulants (EDTA, citrate, or heparin) can introduce variability in the quality and integrity of DNA isolated from blood specimens. The PAXgene Blood DNA Tube† contains an additive that stabilizes DNA without interfering with downstream assays.

Still, many institutions rely on blood collected in tubes without specific sample and analyte stabilizing reagents. The advantage of the PAXgene collection and stabilization products is the preservation of specimen and analyte quality over several storage conditions at various temperatures, which can be proven by the QIAxcel Connect system as a quality control tool [3].

Material and Methods

ccfDNA isolation from blood. Human blood was collected either in PAXgene Blood ccfDNA Tubes (CE-IVD)‡ or in EDTA blood collection tubes. Plasma was generated from either freshly collected blood (<4 h) or from blood stored at 25°C for seven days. Isolation of ccfDNA was performed automatically using the QIAsymphony® DSP Circulating DNA Kit§ on the QIAsymphony SP§ instrument. The concentration of ccfDNA was measured using the Qubit® dsDNA HS assay kit (Thermo Fisher Scientific) on the Qubit 3.0 fluorometer (Table 1).

Table 1. Overview of ccfDNA samples from blood

	Input plasma volume [mL]	Elution volume [µL]	Concentration (ng/µL)	Condition
Sample 1	2	60	0.80	PAXgene Blood ccfDNA Tubes < 4 h
Sample 2.1	2	60	0.27	PAXgene Blood ccfDNA Tubes < 4 h
Sample 2.2	2	60	0.42	PAXgene Blood ccfDNA Tubes 7 d 25°C
Sample 2.3	2	60	0.24	EDTA Tube < 4 h
Sample 2.4	2	60	7.00	EDTA Tube 7 d 25°C

cfDNA isolation from urine. Human urine was collected in the PAXgene Urine Collection Cup (RUO)† and either left unstabilized or stabilized with the PAXgene Urine Liquid Biopsy Tube (RUO)†. The urine was either processed directly (<4 h) or stored at 25°C for three days. Isolation of cfDNA from urine was performed automatically using the QIAsymphony DSP‡ Circulating DNA Kit on the QIAsymphony SP instrument. The concentration of cfDNA was measured using the Qubit dsDNA HS assay kit (Thermo Fisher Scientific) on the Qubit 3.0 fluorometer (Table 2).

Table 2. Overview of cfDNA samples from urine

	Input urine volume [mL]	Elution volume [µL]	Concentration (ng/µL)	Condition
Sample 3	8	60	1.14	PAXgene Urine Liquid Biopsy Tubes < 4 h
Sample 4.1	8	60	2.64	PAXgene Urine Liquid Biopsy Tubes < 4 h
Sample 4.2	8	60	1.01	PAXgene Urine Liquid Biopsy Tubes 3d 25°C
Sample 4.3	8	60	3.98	Unstabilized < 4 h
Sample 4.4	8	60	0.73	Unstabilized 3d 25°C

gDNA isolation from blood. Human blood was collected in PAXgene Blood DNA Tubes (RUO)†. Isolation of gDNA was performed manually using the PAXgene Blood DNA Kit (RUO)†. The concentration of gDNA was measured using the QIAxpert® instrument† (Table 3). All samples were diluted to 50 ng/µL with QIAxcel dilution buffer for the QIAxcel run.

Table 3. Overview of gDNA samples from blood

	Input blood volume [mL]	Elution volume [µL]	Concentration (ng/µL)	Condition
Sample 5	8.5	1000	279.60	PAXgene Blood DNA Tubes < 4 h
Sample 6	8.5	1000	218.90	PAXgene Blood DNA Tubes < 4 h
Sample 7	8.5	1000	304.10	PAXgene Blood DNA Tubes < 4 h

Run parameters. Capillary gel electrophoresis was done using the QIAxcel Connect system [4]. Instrument and analysis software was ScreenGel SW 2.1. The following QIAxcel kits, which contain ready-to-use gel cartridges with nucleic acid staining dye, were used.

For cfDNA analysis: QIAxcel DNA High Sensitivity kit with 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 – 1 kb (provided with QIAxcel DNA High Sensitivity Kit). For analysis of cfDNA from urine, the size end range of the size marker was manually set to 5000 bp in the ScreenGel software.

For gDNA analysis: QIAxcel DNA Screening Kit with run method AM1800 (available as customized method), Alignment Marker QX 15 bp and Size Marker QX DNA Large-Fragment 1 kb – 20 kb.

Results and Discussion

ccfDNA from blood

ccfDNA typically has a predominant fragment size range of around 166–170 bp. This corresponds to the length of DNA wrapped around a nucleosome (147 bp), plus an additional stretch to link two nucleosome cores. Apoptosis can also produce longer ccfDNA fragments that correspond to di- (~350 bp), tri- (~565 bp) or poly-nucleosomes [5]. Figure 1 shows the electropherogram of isolated ccfDNA (sample 1, 0.80 ng/µL) from human plasma generated from blood collected into PAXgene Blood ccfDNA Tubes. The mono-nucleosomal peak and the di-nucleosomal peak are detected. The absence of high molecular weight genomic DNA indicates proper sample stabilization.

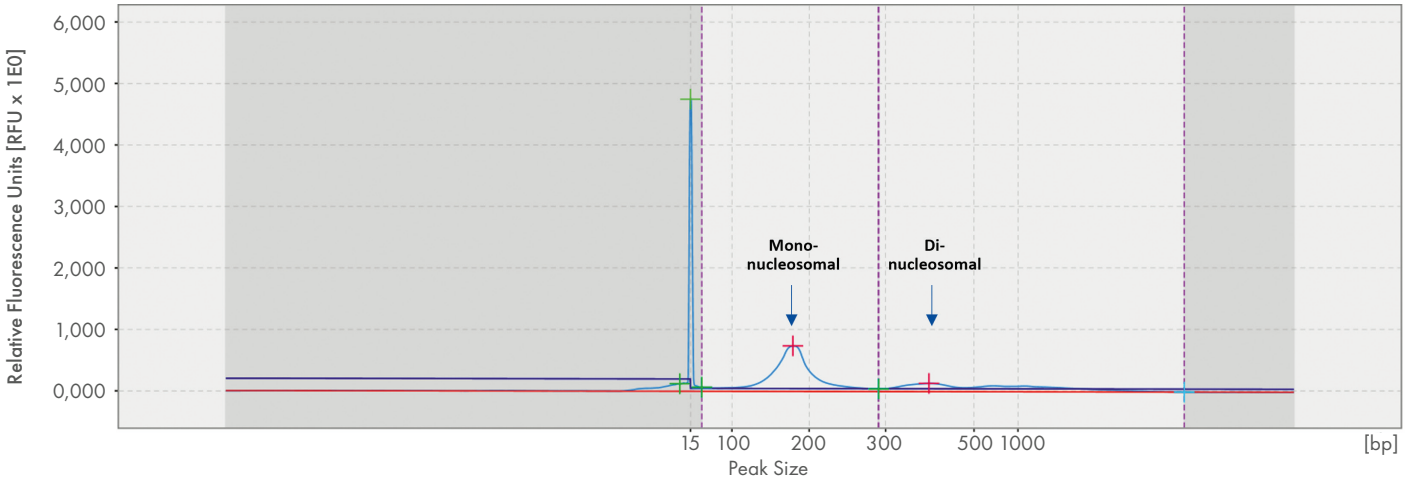
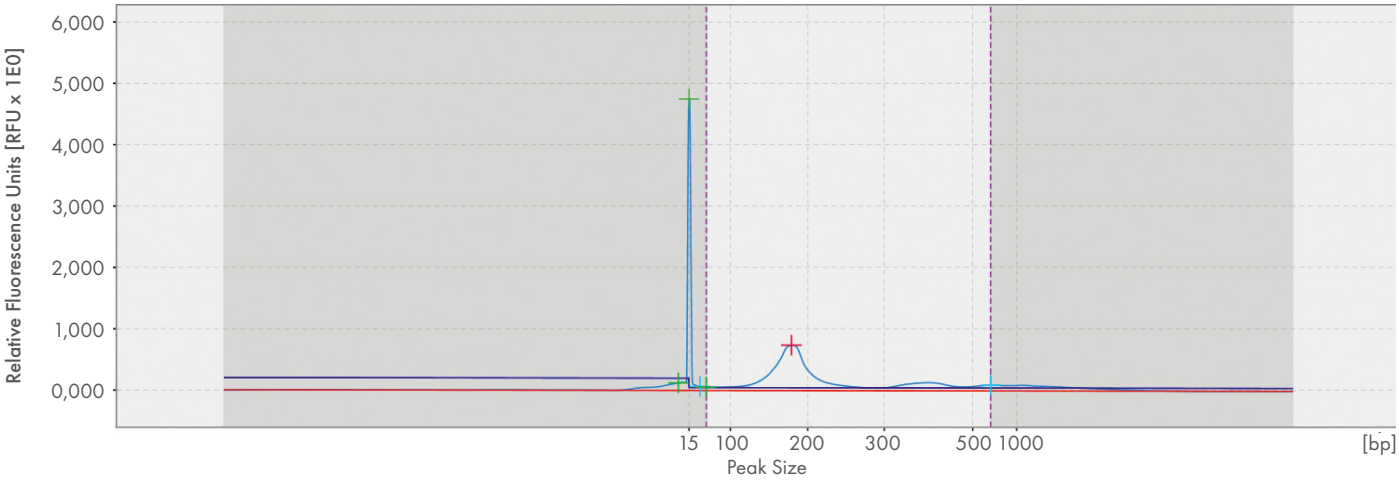


Figure 1. Electropherogram of isolated ccfDNA (Sample 1) from plasma of blood collected into a PAXgene Blood ccfDNA Tube.

With the distribution analysis feature of the QIAxcel ScreenGel Software, it is possible to calculate the ratio between an area of interest and total sample. In Figure 2 the area of interest from the electropherogram shown in Figure 1 is set to 50 bp – 700 bp (Sample 1). The area of interest was computed at 0.87 which means that 87% of the sample is within this size range. The remaining 13% is DNA of higher molecular weight outside of this size range.



	Area of Interest			ccfDNA		
Sample Info	Mol [pmol/l]	Size Start [bp]	Size End [bp]	Numerator	Denominator	Ratio (Concentration)
Sample 1	4423,46	50	704	Area of Interest	Total Sample	0,87

Figure 2. Ratio calculation between the area of interest and the total sample for Sample 1.

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 [6]. Figure 3 shows that the PAXgene Blood ccfDNA Tube stabilization solution helps to prevent cell lysis and release of gDNA into plasma. Samples collected in PAXgene Blood ccfDNA Tubes and EDTA tubes were processed within 4 h after collection (T_0, green and red graphs) or after storage for seven days at 25°C (T_7, lilac and blue graphs). In the T_7 EDTA sample (blue graph), various fragments were observed resulting from severe gDNA release in the absence of a stabilization solution. This effect was not observed in the matched PAXgene Blood ccfDNA Tube sample (lilac graph).

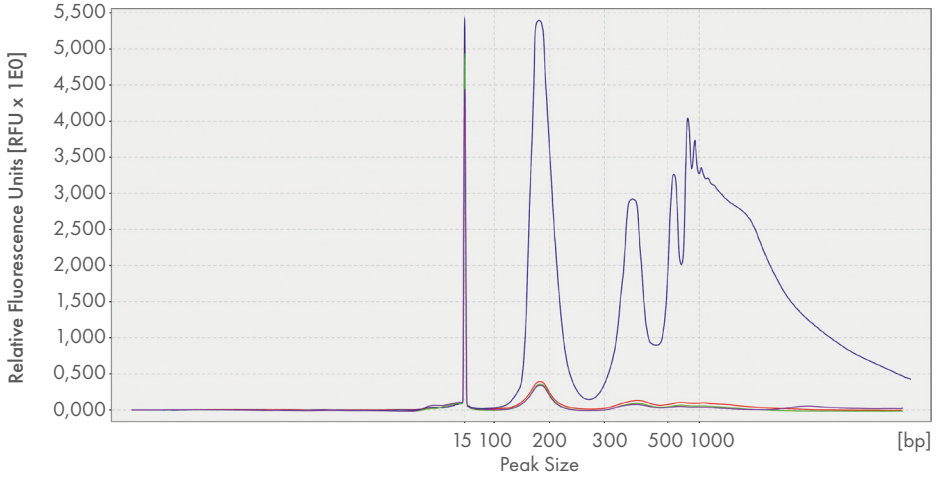


Figure 3. Superimposed view of the electropherograms at T_0 – PAXgene Blood ccfDNA Tube (green), T_7 – PAXgene Blood ccfDNA Tube (lilac), T_0 – EDTA Tube (red) and T_7 – EDTA Tube (blue) (Sample 2.1 – 2.4).

cfDNA from urine

Urine cfDNA shows a different sizing than blood ccfDNA. In addition to mono- and di-nucleosome patterns, longer fragments can be detected [7]. Figure 4 shows a typical electropherogram of cfDNA (sample 3, 2.80 ng/µL) isolated from urine stabilized with the PAXgene Urine Liquid Biopsy Tube and isolated on the day of urine collection.

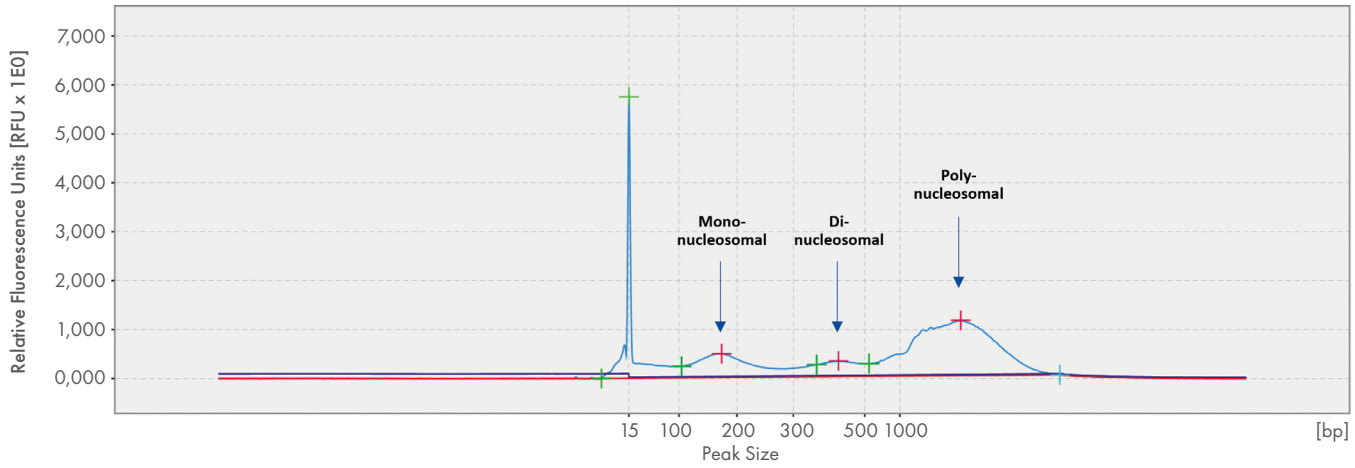


Figure 4. Electropherogram of cfDNA (sample 3) isolated from urine collected and stabilized with the PAXgene Urine Liquid Biopsy Set.

Figure 5 shows the difference in peak pattern of cfDNA isolated from unstabilized urine (blue and lilac graphs) and from urine stabilized with the PAXgene Urine Liquid Biopsy Tube (red and green graphs). The nuclease activity in urine is higher than in blood. DNA gets rapidly degraded in unstabilized urine prohibiting the detection of larger cfDNA fragments. cfDNA is clearly more fragmented in unstabilized urine compared to stabilized urine already if cfDNA is isolated on the day of urine collection without any storage (< 4 h after collection, compare blue and red graphs). After storage of urine at 25°C for three days, only degraded cfDNA can be isolated from unstabilized urine (lilac graph). cfDNA isolated from urine stored in the PAXgene Urine Liquid Biopsy Tube at 25°C for three days shows a similar cfDNA profile than for urine stabilized and not stored before cfDNA isolation.

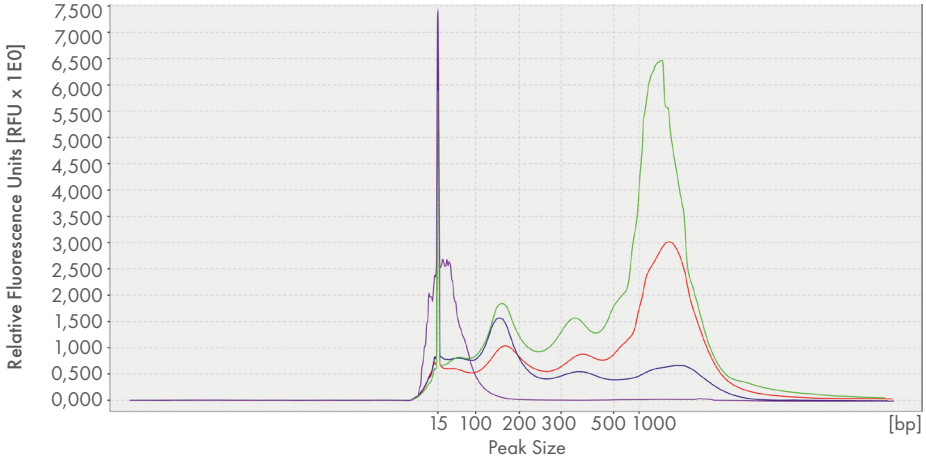


Figure 5. Superimposed view of the electropherograms of urine cfDNA from one individual at T_0 – PAXgene Urine Liquid Biopsy Tube (red), T_3 – PAXgene Urine Liquid Biopsy Tube (green), T_0 – unstabilized (blue) and T_3 – unstabilized (lilac).

gDNA from blood

Inconsistent blood draw volumes and different anticoagulants can introduce variability into the quality and integrity of DNA isolated from blood specimens. Figure 6 shows that the gDNA, isolated from PAXgene Blood DNA Tubes, has a very comparable profile regarding, size, shape and integrity between three different individuals (Sample 5 – Sample 7). The size of the DNA is > 20 kb, which indicates high molecular weight DNA.

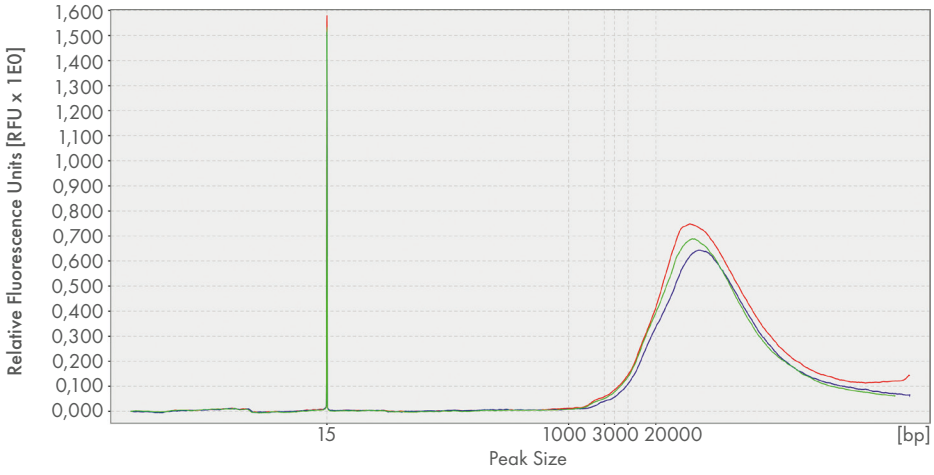


Figure 6. Superimposed view of the electropherograms of gDNA from three different samples isolated from PAXgene Blood DNA Tubes representing three different individuals. Sample 5 (red), sample 6 (blue), sample 7 (green).

Conclusion

PAXgene products are specifically developed to integrate and standardize workflows for sample collection, stabilization and purification of high-quality nucleic acids from human samples. This enables flexible transport and storage conditions while providing high-quality samples for downstream analyses.

Blood and urine are common body fluids that are used for the isolation of nucleic acids, given their potential in providing complementary results for a more comprehensive understanding of a disease profile. The quality of the isolated nucleic acids is critical for downstream applications of the samples.

In this application note, the advantage of the PAXgene collection and stabilization products on the preservation of specimen and analyte quality has been proven by the QIAxcel Connect system. In cfDNA samples from blood, cell lysis and gDNA release is minimized. In cfDNA samples from urine, the degradation of cfDNA and the release of gDNA is clearly minimized. gDNA from blood of different individuals shows a very comparable DNA profile between each other.

References

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6. Nagata, S., Apoptotic DNA Fragmentation, 2000. 256(1): p.12-18
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Ordering Information

Product	Contents	Cat. no.
PAXgene Blood ccfDNA Tube (CE-IVD) (100)‡	100 PAXgene Blood ccfDNA Tubes (10 mL)	768165
PAXgene Blood ccfDNA Tube (RUO)†	100 PAXgene Blood ccfDNA Tubes (10 mL)	768115
PAXgene Urine Liquid Biopsy Set (20)†	20 tubes: PAXgene Urine Liquid Biopsy Tube (RUO, sterile, 10.0 mL draw volume, 1.5 mL additive) 20 cups: PAXgene Urine Collection Cup (RUO, sterile, up to 120 mL urine volume)	769143
PAXgene Blood DNA Tubes (RUO) (100)†	100 PAXgene Blood DNA Tubes	761115
PAXgene Blood DNA Kit (RUO) (25)†	Processing tubes and buffers for 25 preparations	761133
QIAsymphony DSP Circulating DNA Kit (192)§	Includes Reagent Cartridges, accessories and Proteinase K vials for 192 preps of 2000 µL or 4000 µL or 384 preps of 1000 µL sample volume.	937556
QIAsymphony SP¶	For fully automated DNA/RNA purification from a broad range of samples with varying input volumes, with the QIAsymphony SP, and integrated assay set up with the QIAsymphony AS.	9001751
QIAxcel Connect system†	Effortless, cost-effective, high-resolution DNA or RNA gel electrophoresis – all in a single instrument. Capillary electrophoresis device: includes computer, QIAxcel ScreenGel Software, and 1-year warranty on parts and labor.	9003110
QIAxcel DNA High Sensitivity Kit (1200)†	For automated analysis of DNA fragments using QIAxcel instruments. QIAxcel DNA High Sensitivity Cartridge, QIAxcel DNA High Sensitivity Marker Set, Buffers, Mineral Oil, 12-Tube Strips	929012
QIAxcel DNA Screening Kit (2400)†	For automated analysis of DNA fragments using QIAxcel instruments. QIAxcel DNA Screening Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004

* Products and product claims may differ from country to country based on regulations, clearances and approvals and may not be available in certain countries. Please visit www.preanalytix.com or contact your local supplier or country representative for further details.
† For research use only. Not for use in diagnostic procedures.
‡ CE marked for In Vitro Diagnostic Use according to Regulation (EU) 2017/746 on In Vitro Diagnostic Medical Devices (IVDR). Not available in all countries.
§ For In Vitro Diagnostic Use.
¶ The QIAsymphony SP is intended to be used in combination with QIAsymphony Kits indicated for use with the QIAsymphony SP for the applications described in the handbook.

For more information on QIAxcel Connect visit: **www.qiagen.com/qiaxcel-paxgene**