APPLICATION NOTE

# LabChip® GX Touch™ cfDNA Assay for High Throughput Analysis of Cell-free DNA

LabChip® GX Touch™ Nuleic Acid Analyzer


AUTHORS

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## Abstract

A microfluidic assay was developed to analyze cell-free DNA (cfDNA) samples using LabChip® GX Touch™ Nucleic Acid Analyzer. Unlike many other assays which use a higher base molecular weight upper marker for DNA quantitation, the cfDNA assay uses a 50 base pair DNA as an internal standard that is similar in size to cfDNA smears. This analysis method corrects sample injection bias enabling improved quantitation accuracy. Faster analysis and higher assay sensitivity is achieved by using optimized gel matrix conditions. The LabChip® cfDNA Assay is capable of detecting cfDNA samples at as low as 25 pg/µL concentration (S/N>3) and has a linear range of 50-1000 pg/µL. Quantitation accuracy within ±20% of the target concentration and sizing accuracy within ±10% target can be achieved. These accuracy statistics were generated using 6-sample plates with extracted cfDNA of varying concentrations using two commercial extraction kits. The new assay combines the high-throughput functionality of the LabChip GX Touch Nucleic Acid Analyzer with a more robust and tailored assay for cfDNA analysis.


***Table 1:*** *Comparison of Assay Specifications of LabChip cfDNA Assay and Competitor A Assay (closest in performance available in the market)*

| Specifications | LabChip cfDNA | Competitor A |
|---|---|---|
| Sizing Range | 50–7000 bp | 50–800 bp |
| Sizing Accuracy | ±10% | ±15% |
| Sizing Precision | 5% C.V. | 10% C.V. |
| Sensitivity | 25 pg/µL | 20 pg/µL |
| Conc. Accuracy | ±20% | ±20% |
| Conc. Precision | 15% C.V. | 15% C.V. |
| Speed | 90 min/96 samples | 150 min/96 samples |
| Minimum Volume | 1 µL | 2 µL |



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## Introduction

Fast, automated, and high throughput platforms for the separation and analysis of biomolecules have high demand in the biotherapeutic and genomics market. PerkinElmer's LabChip GX Touch nucleic acid analyzer meets those demands by providing rapid electrophoretic separation with accurate nucleic acid quantitation. The reusable microfluidic chip and instrument are designed to analyze minimal sample input volumes via automated sample sipping, perform on-chip fluorescent labeling, and enable high-throughput analysis with runtime as low as 40 seconds per sample. LabChip GX Touch nucleic acid analyzer offers multiple assays to assess genomic DNA and RNA integrity, DNA smear analysis for NGS libraries, and DNA/ RNA fragment analysis for PCR fragments. The new LabChip cfDNA assay is developed for the analysis of cfDNA samples extracted from plasma using different commercially available extraction kits.

cfDNA refers to free circulating DNA fragments and is emerging as an important biomarker for less invasive 'liquid biopsy' applications[1, 2]. The extraction and next-generation sequencing of cfDNA are currently being developed for diagnostic purposes[3]. An important upstream quality control check is confirmation of the quality of the extracted cfDNA sample, its concentration as well as the distribution of mono, di, and tri-nucleosomal fractions of the DNA sample. cfDNA samples are commonly analyzed on microfluidic platforms, including the LabChip GX Touch Nucleic Acid Analyzer and other systems with a limit of detection as low as 50 pg/µL obtainable on PerkinElmer NGS 3K Assay[4]. A common problem, however, is that carry-over of salt or other organic/biological species in the extracted sample may lead to decreased upper marker signal or complete disappearance of the upper marker peak resulting in inaccurate quantitation and sizing of cfDNA. To address this gap, we introduced a 50 bp dsDNA internal marker for cfDNA quantitation. We also optimized the gel sieving matrix to provide baseline resolution of the mono and di-nucleosomal cfDNA peaks with maximal mobility (assay speed) and sensitivity. The concentration accuracy and precision of the cfDNA assay are similar to or better than the commercial competitor methods. Our accuracy statistics were generated using 6 X 96-sample plates with extracted cfDNA of varying concentrations using two commercial extraction kits. We also demonstrate that the LabChip cfDNA assay can be used for the analysis of cfDNA samples prepared from multiple commercial extraction kits.

## Experimental

cfDNA samples were extracted from pooled plasma reformatted from whole blood tubes by Janus Blood iQ using different extraction kits [chemagic™ cfDNA 1.5k Kit (PerkinElmer, Part # CMG-1396), MagMAX™ Cell-Free DNA Isolation Kit (Thermo Fisher Scientific, Part # A29319), chemagic™ cfDNA 5k Kit (PerkinElmer, Part # CMG-134), QIAamp® Circulating Nucleic Acid Kit (Qiagen, Part # 55114)] according to manufacturer-recommended protocols. The LabChip GX Touch Nucleic Acid Analyzer consists of an instrument and two consumables (reagent kit and microfluidic chip). Gel dye matrix was prepared by adding 13 µL of cfDNA dye concentrate to 1.1 mL of Gel matrix and mixed. The vial was capped, inverted 10X, vortexed for 20 s, and transferred to 2 spin vials. The spin vial tubes were centrifuged at 9300 rcf for 7.5 minutes at room temperature. Using a reverse pipetting technique, the gel-dye mixture was pipetted into the wells 3,7, 8, and 10 of the LabChip® with amounts indicated in Figure 1A. Meanwhile, cfDNA 1X marker solution was prepared by diluting 300 µL of the 10x cfDNA Marker to 3 mL using DI water followed by mixing with 60 µL of marker booster solution. cfDNA samples were diluted 10-fold using the cfDNA 1X marker solution containing sizing markers and 50-bp internal standard on individual wells in 98 or 384 well plates. Sample (~500 nL) is then sipped onto the chip from 96 or 384-well plates and injected into the separation channel. Electrokinetic separation of mono, di and tri-nucleosomal fragments are detected using laser-induced fluorescence.

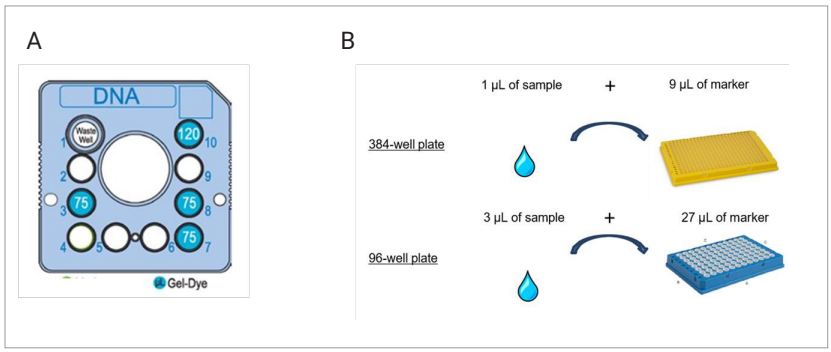


***Figure 1: A)*** *LabChip well caddy indicating the well positions where dye reagent is loaded for mixing with nucleic acid samples. In wells 3,7,8 and 10, gel-dye matrix is loaded.* ***B)*** *Schematic for simple cfDNA sample preparation for cfDNA analysis. A 1-3 µL sample is mixed with a marker solution that contains both a sizing marker and a 50 bp dsDNA quantitation marker.*

## Results & Discussion

### Assay Accuracy with Competitor Method

To demonstrate assay performance, the concentration and size of cfDNA samples extracted with Extraction kit A were first determined by analyzing the samples with an electrophoresis-based reference method (Competitor method A). The accuracy of the LabChip® GX Touch™ cfDNA assay for sizing and concentration of cfDNA was then determined by running the same samples on LabChip GX Touch Nucleic Acid Analyzer with cfDNA assay (on different chips and different instruments). Representative results from both platforms are presented in the electropherogram below, Figures 2A and 2B.

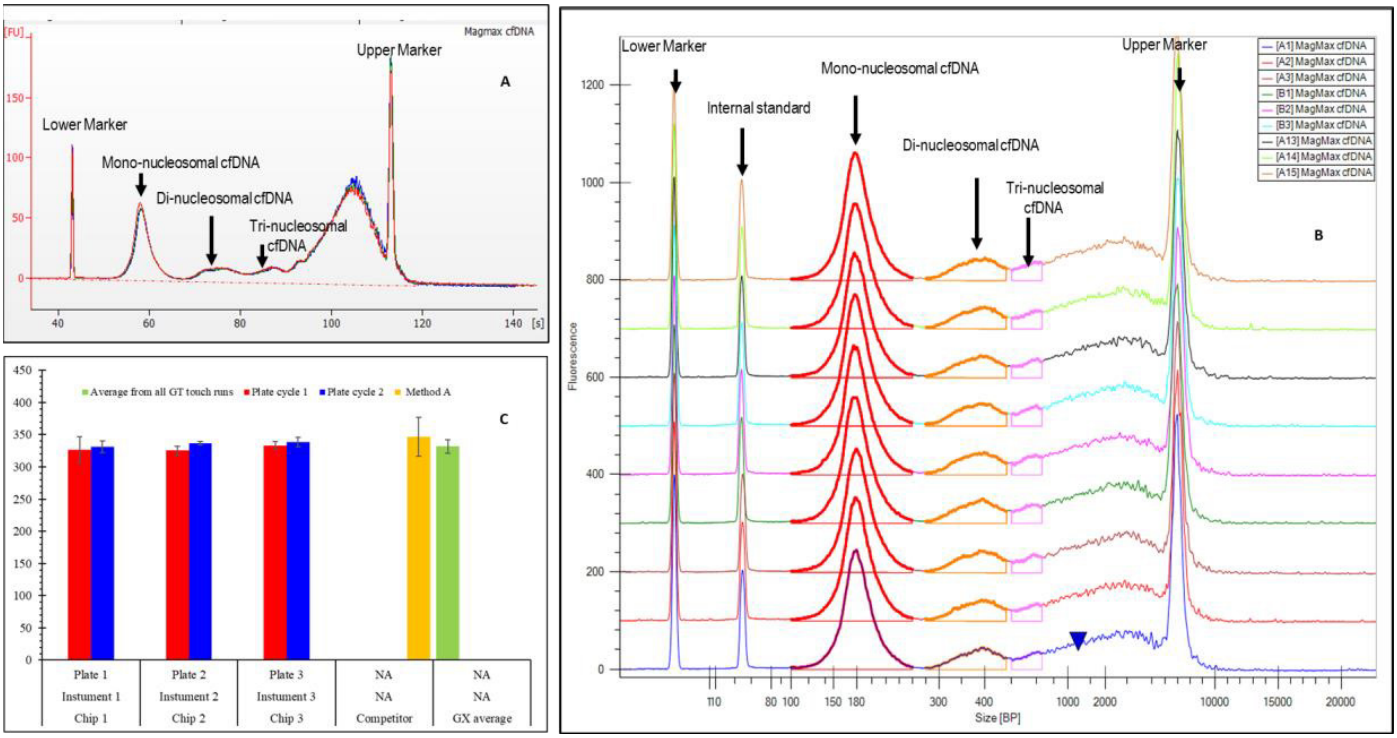


***Figure 2: A)*** *Electropherogram of the cfDNA sample measured by reference method (competitor method A).* ***B)*** *Stacked Electropherogram obtained for cfDNA sample plated in 3 wells sipped in triplicate in a plate.* ***C)*** *Bar graph representing the average (n=9) concentration of mono-nucleosomal cfDNA measured by LabChip cfDNA assay on different chips and instruments (2 plate cycles) and competitor method A. The average of all LabChip measurements (n=54) is compared with the average of competitor method A (n=9)*

Data obtained using competitor method A and LabChip cfDNA assay were analyzed and are presented as bar graphs (see Figure 2C) and in Table 2. The LabChip GX Touch Nucleic Acid Analyzer runs were carried out on 3 different instruments with 3 different chips; each plate was cycled twice to test the accuracy and precision of the analysis. As observed from the analyzed data of individual plate cycles (average of 9 sips of each sample) as well as the average of the whole population, the concentration of cfDNA (mono-nucleosomal peak) obtained from LabChip GX Touch Nucleic Acid Analyzer is in close agreement for concentration and sizing with the competi-tor method (quantitation accuracy within ±5% variation and sizing accuracy within ±1% considering the concentration obtained from competitor method as true value).

***Table 2:*** *Average concentration and size of mono-nucleosomal cfDNA with respective CV measured by LabChip cfDNA assay on different chips and instruments (2 plate cycles) and competitor method A.*

| Chip | Plate | Instrument | Plate Cycle | Av. Conc. (n=9) (pg/µL) | Conc RSD | Av. Size (n=9) (Base pair) | Size RSD |
|---|---|---|---|---|---|---|---|
| Chip 1 | Plate 1 | Instrument 1 | Plate cycle 1 | 326.3 | 6.6 | 176 | 0.6 |
| Chip 1 | Plate 1 | Instrument 1 | Plate cycle 2 | 331.4 | 3.0 | 175 | 0.5 |
| Chip 2 | Plate 2 | Instrument 2 | Plate cycle 1 | 325.5 | 2.3 | 178 | 0.8 |
| Chip 2 | Plate 2 | Instrument 2 | Plate cycle 2 | 336.5 | 1.0 | 176 | 0.9 |
| Chip 3 | Plate 3 | Instrument 3 | Plate cycle 1 | 332.9 | 2.1 | 177 | 0.9 |
| Chip 3 | Plate 3 | Instrument 3 | Plate cycle 2 | 338.4 | 2.3 | 175 | 0.8 |
| Whole population | NA | NA | Average | 331.8 | 3.5 | 176 | 0.9 |
| **Competitor** | **NA** | **NA** | **Method A** | **346.5** | **8.7** | **175** | **1.1** |

As seen in Table 2, our assay measured the average mono-nucleosomal cfDNA concentration at 331.8 pg/µL (RSD ±3.5) across different instruments and chips for the same sample measured at 346.5 pg/µL (RSD ±8.7) by competitor method. Similarly, the LabChip GX Touch Nucleic Acid Analyzer measured an average mono-nucleosomal cfDNA size as 176 bp (RSD ±0.9) while the competitor method measured 175 bp (RSD ±1.1). Both methods perform sizing measurements within several base pair numbers to the cfDNA sizes published in the literature (peak maximum at 167 bp)[5].

### Analysis Time, Sensitivity, & Linear Range

Each sample run is completed in 40 s with LabChip cfDNA assay as seen in the electropherogram in Figure 3A, (2.5 times faster compared to the competitor method); a total of 96 samples can be analyzed in 1 hour and 26 minutes (instrument warm-up time included) in one chip preparation in high-throughput mode. Because of the optimized gel formulation, a higher signal is achieved for cfDNA smear resulting in a high signal-to-noise ratio and improved assay sensitivity (LOD<25 pg/µL, S/N>3). We tested the linear range of our assay for quantitation of cfDNA using a serial dilution of cfDNA samples, and the assay shows excellent linearity (R2 >0.99) between theoretical and experimental concentration, as seen in the plot in Figure 3B.

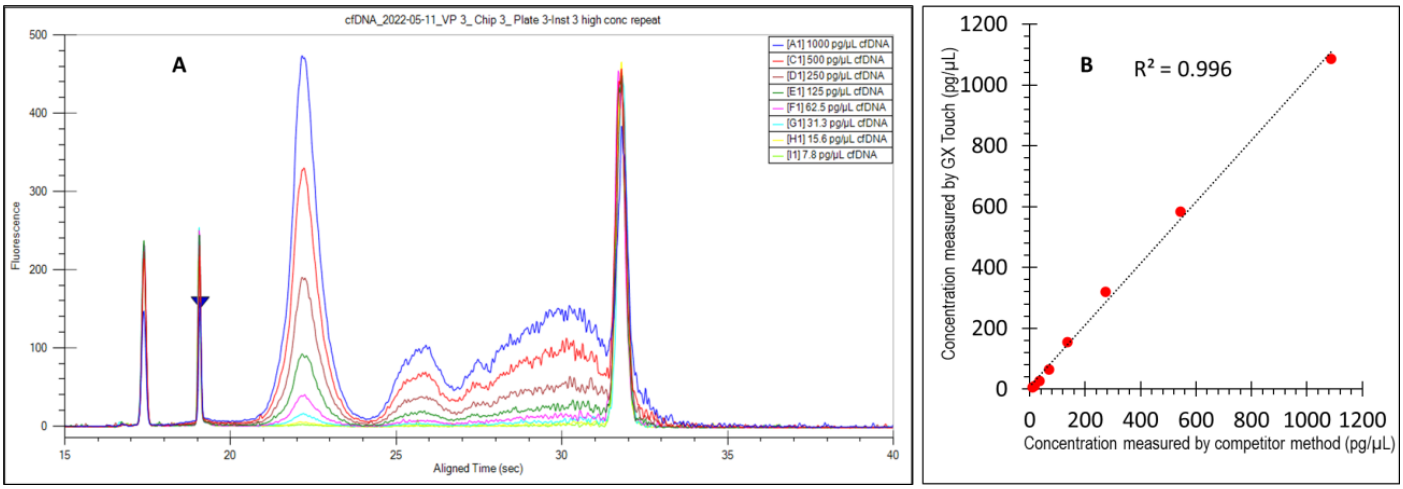


***Figure 3: A)*** *LOD and linearity test for the cfDNA assay* ***B)*** *Quantification of a cfDNA sample in dilution series from 50-1000 pg/µL: electropherogram showing the cfDNA smear with serially diluted sample B) plot of mono-nucleosomal cfDNA concentration measured by reference method (competitor method A) vs. Labhip GX Touch instrument.*

### Compatibility with Extraction Kits

Due to different chemistries/reagents used in different extraction kits for extraction of cfDNA, the extracted sample may have different ionic strengths (due to salt content) and impact electrokinetic sample injection. This causes a difference in the signal intensity of cfDNA sample peaks, 50 bp dsDNA internal standard, & upper marker between samples extracted with different kits. Upper marker peak intensity is severely impacted for cfDNA samples extracted with some kits with competitor assays causing inaccurate quantitation and sizing possibly due to injection bias and extraction carryover. cfDNA sample is extracted using three commercial cfDNA extraction kits, chemagic cfDNA 1.5k Kit, QIAamp® Circulating Nucleic Acid Kit, ThermoFisher's MagMAX® Cell-Free DNA Isolation Kit following manufacturer recommended protocol (using different plasma volumes). Extracted samples were diluted with the required volume of TE buffer to normalize to per mL plasma input. Those samples are analyzed by cfDNA assay and results are presented in Figure 4 and Table 1. As evident from the data, quantitation, and sizing by LabChip cfDNA assay are not impacted by cfDNA extraction carryovers and can be used for analyzing cfDNA samples irrespective of the extraction kit used for extraction.

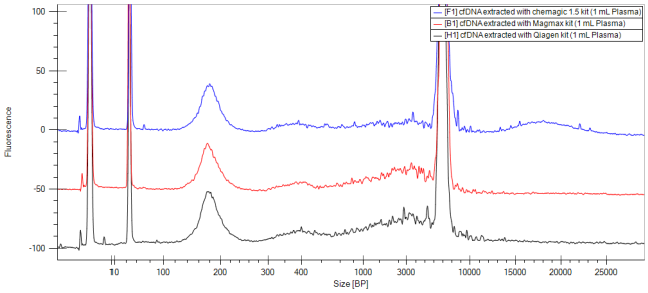


***Figure 4:*** *Overlaid electropherogram of cfDNA samples extracted using chemagic 360, Qiagen, and Magmax extraction kit*

***Table 1:*** *Concentration of mono-nucleosomal cfDNA concentration in samples extracted with different extraction kits analyzed using cfDNA assay (normalized to per mL plasma input)*

| Extraction kit | mono-nucleosomal cfDNA concentration |
|---|---|
| chemagic™ cfDNA 1.5k Kit (PerkinElmer) | 70.8±4.2 |
| QIAamp® Circulating Nucleic Acid Kit (Qiagen) | 61.5±4.7 |
| MagMAX® Cell-Free DNA Isolation Kit (Thermofisher) | 60.1±5.3 |

## Conclusion

In conclusion, we have developed a new assay for the analysis of cfDNA samples. This robust, high-throughput assay can analyze 96 samples in one chip preparation in 1.5 hours. LabChip cfDNA assay offers sensitivity with a higher cfDNA signal smear relative to the competitor method (<25 pg/µL, S/N>3) with a linear dynamic range of 50 pg/µL- 1000 pg/µL for quantitation of cfDNA. Additionally, LabChip cfDNA assay can be used for the analysis of cfDNA samples irrespective of the extraction kits used.

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### References

1. *Norton, M. E. et al. Cell-free DNA Analysis for Noninvasive Examination of Trisomy. N. Engl. J. Med. 372, 1589–1597 (2015).*
2. *Corcoran, R. B. & Chabner, B. A. Application of cell-free DNA analysis to cancer treatment. N. Engl. J. Med. 379, 1754–1765 (2018).*
3. *Wan, J. C. M. et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer. 17, 223-238 (2017).*
4. *DNA NGS 3K Assay Quick Guide, PerkinElmer Inc. https://www.perkinelmer.com/product/ht-dna-ngs-3k-reagent-kit-cls960013*
5. *Alcaide, M. et al. Evaluating the quantity, quality and size distribution of cell-free DNA by multiplex droplet digital PCR. Sci Rep 10, 12564 (2020).*

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