APPLICATION NOTE

LabChip® GX Touch™ Nucleic Acid Analyzer

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NGS 3K Assay for High Sensitivity Measurement of Cell-free DNA

Summary

The high sensitivity and throughput of the NGS 3K assay for the LabChip® GX Touch™ nucleic acid analyzer allows for the assessment of size, purity, and quantity of cell-free DNA (cfDNA) for use in applications requiring robust quality control. There are several commercial kits available for isolating cfDNA, with each employing proprietary buffer chemistries optimized for the extraction method, which may be bead- or column-based. Carry-over contaminants (salts, proteins, or other) from these commercial kits can result in poor performance in electrophoresis assays. To address this, PerkinElmer has developed an easy and optimized method for producing high quality data from these "hard to analyze" cfDNA samples. The method described here significantly improves the robustness and repeatability of the NGS 3K assay for cfDNA samples.

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Background

PerkinElmer's LabChip® GX Touch™ nucleic acid analyzer utilizes microfluidic separation and measurement of nucleic acids with industry-leading sensitivity and throughput. The quartz microfluidic chip and instrument are designed to analyze minimal sample input volumes, perform on-chip fluorescent labeling, and enable high-throughput assays via automated sample-sipping. The NGS 3K assay is PerkinElmer's most sensitive nucleic acid analysis assay designed to analyze DNA for NGS library preparation and NGS libraries prior to sequencing. The NGS 3K assay is able to provide quantitation of DNA fragments and smears in concentration ranges from 5-5000 pg/µL and 50-5000 pg/µL, respectively. For these precious samples, minimizing the volume required for analysis is of paramount importance, which is why the NGS 3K assay and the LabChip® GX Touch™ nucleic acid analyzer are the perfect tools to analyze these samples.

NGS 3K Protocol for Cell-free DNA Samples

This optimized protocol uses the proprietary NGS 3K Marker Booster ("Marker Booster"), included in the NGS 3K reagent kit, with the marker solution which is added to cell-free DNA samples. The Marker Booster has been formulated to compensate for changes in the ionic strength and pH of the sample solution, which allows for increased recovery and improved repeatability of the upper marker signal. Given that the upper marker is used as an internal standard during the sample concentration calculations, upper marker signal stability is of critical importance to NGS 3K assay performance. By stabilizing the signal of the upper marker, precision, accuracy, and repeatability are maximized. The Marker Booster may be added to the marker solution immediately prior to sample dilution (1:27 ratio) or directly to the sample marker mixture (1:30 ratio), whichever method is more easily integrated into existing workflows.

To achieve the results shown below, 1 µl of NGS 3K Marker Booster was added directly to prepared NGS 3K cfDNA samples, as indicated, and all samples were then run on the LabChip® GX Touch™ nucleic acid analyzer using the standard NGS 3K assay.

Results

Samples

cfDNA samples can show upper marker signal variability, manifesting as an upper marker signal less than lower marker signal. A series of 64 cell-free DNA samples isolated using four commercial cell-free DNA extraction kits (Kits A-D) were analyzed using the NGS 3K assay with the Marker Booster. Each sample was prepared with and without the Marker Booster according to the assay insert, summarized above, and was run using the default NGS 3K assay software settings with 2 plate cycles.

Robustness of Upper Marker Signal

The set of samples was previously measured using the NGS 3K assay where many samples showed a loss of upper marker signal and poor quantitation CV. After addition of the NGS 3K Marker Booster, all samples achieved upper marker signal stabilization, with an expected peak height greater than the lower marker signal. A representative electropherogram is shown in Figure 1 and demonstrates the recovery of the upper marker in cfDNA extracted using four different DNA extraction kits.

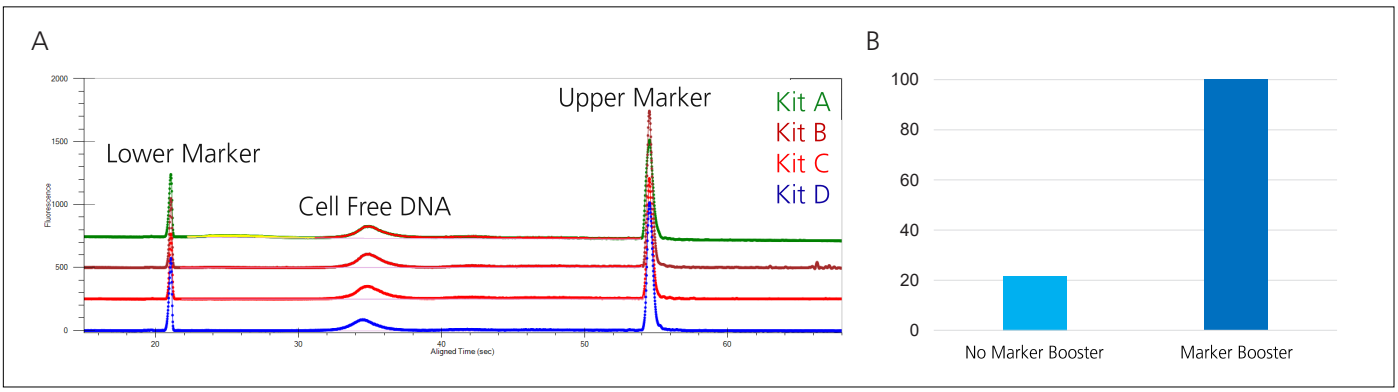

Figure 1. A) Representative electropherograms of cell-free DNA samples using 4 different commercial cfDNA extraction kits A-D. B) Shows the percentage of cell-free DNA samples tested showing upper maker signal recovery (n=64).

Concentration CV and Accuracy in the Presence of Marker Booster

The variation of sample CVs for cfDNA isolated using each commercial cell-free DNA extraction kit is shown in Figure 2A. The NGS 3K CV specification of 20% criteria is met across all solutions. The average CV for cfDNA isolated using each commercial kit is less than 10%, with all samples showing CV less than 20%. This demonstrates that the upper marker signal has a high degree of repeatability for samples purified with these cell-free DNA extraction kits when the Marker Booster is used in the NGS 3K sample preparation.

The concentration accuracy comparison of the optimized NGS 3K assay and the Thermo Fisher® Qubit® assay using extracted cell-free DNA samples is shown in Figure 2B. We found that 80% of samples are within 30% of the concentration value obtained by Thermo Fisher® Qubit® high sensitivity assay, which was greater than the ~±15% reported concentration accuracy error for the Thermo Fisher® Qubit® high sensitivity assay.[1] A potential reason for the discrepancy is the fluorescent signal in Thermo Fisher® Qubit® assay is dependent on salt concentration, which could be influenced by the buffer compositions of DNA extraction kits.[2] Additionally, the Thermo Fisher® Qubit® assay also measures the total DNA concentration in the sample, which may include high or low molecular weight impurities or aggregates, whereas the NGS 3K assay quantifies the cell-free DNA fraction only.

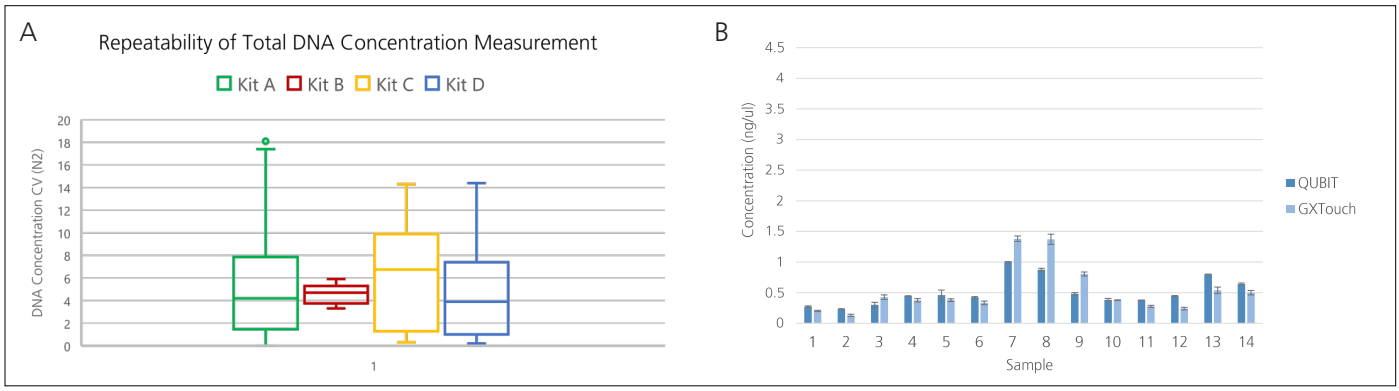

Figure 2. A) Box and whisker plot for concentration CVs. Each CV is calculated from two replicates of cfDNA from commercial DNA extraction kits A (38 samples)-Green, B (6 samples)-Red, C (6 samples)-Orange, and D (14 samples)-Blue. All commercial kits showed a median CV value less than 10%. B) The concentration of DNA extracted from cell-free samples (ng/µL) was measured using the optimized NGS 3K method and compared to measurements obtained with the Thermo Fisher® Qubit® high sensitivity assay. Each sample was tested in triplicate.

Conclusion

The PerkinElmer NGS 3K assay for the LabChip® GX Touch™ nucleic acid analyzer offers a simple and robust solution for the quantitative measurement of cell-free DNA. Here we've shown the Marker Booster maintains the sensitivity, accuracy, and repeatability of the NGS 3K assay by stabilizing the signal of the upper marker in cfDNA extracted with four different cell-free DNA extraction kits, proving PerkinElmer's NGS 3K assay is a valuable addition to NGS and genomics workflows.

References

1. Comparison of Quant-iT™ and Qubit® DNA quantification assays for accuracy and precision, Application Note, Thermo Fisher.
2. Emission Characteristics of Fluorescent Labels with Respect to Temperature Changes and Subsequent Effects on DNA Microchip Studies, Wen-Tso Liu et.al., Applied and Environmental Microbiology, 2005 Oct; 71(10): 6453–6457.

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