APPLICATION NOTE

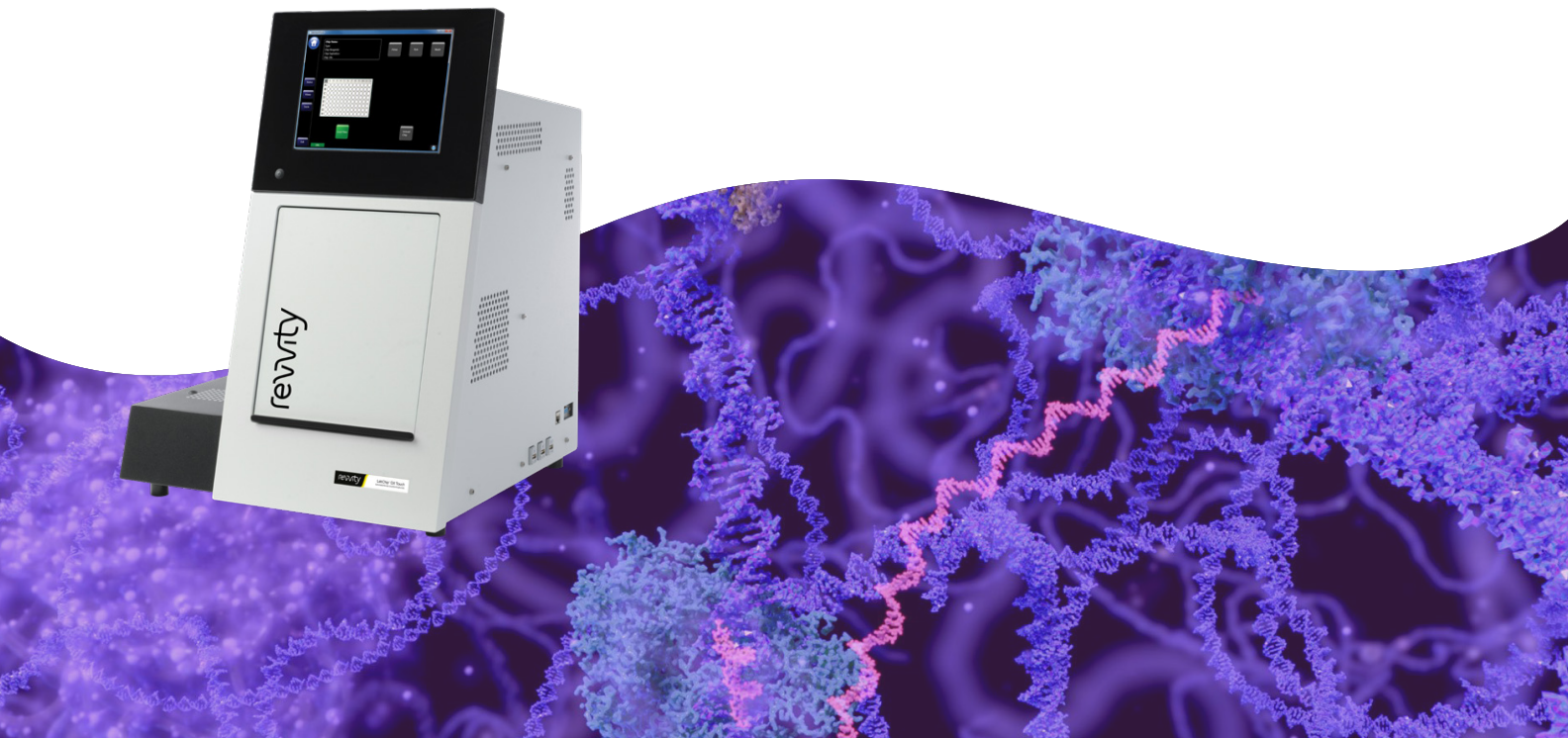
LabChip GX Touch small RNA assay.

For research use only. Not for use in diagnostic procedures.

Introduction

Rapid and automated electrophoresis is an important tool for research and development of new biotherapeutics. The LabChip® GX Touch™ Nucleic Acid Analyzer performs automated microfluidic capillary electrophoresis on reusable chips with analysis times up to 65 seconds per sample. Microfluidic capillary electrophoresis provides industry leading throughput with accurate sizing and quantitation results. Sample types including cell free DNA, genomic DNA, RNA and proteins may all be characterized on the LabChip® GX Touch™ system by running the appropriate reagent kit and software package.

The new LabChip® Small RNA assay was developed for analysis of small RNAs 20-150 nucleotides (nt) long. The low molecular weight size range can be used for CRISPR/Cas workflows as a quality control check point of synthetic guide RNA (gRNA). The assay also simplifies purity analysis of RNA-targeting therapeutics. Like slab gel electrophoresis protocols published for small RNA analytes that use high agarose cross-linking content and high concentration of urea, the new LabChip® Small RNA assay uses a urea containing gel formation with pore size optimized for small RNA detection. The GX Reviewer settings for small RNA analysis allows fragment analysis of small RNA molecules analogous to the method used for LabChip® High Sensitivity DNA assays.



Assay overview and performance

The newly developed gel matrix and ladder standard provide a system for the separation of small RNA molecules. An example ladder trace is shown in Figure 1. The ladder consists of 5 fragment oligonucleotides to generate a sizing calibration curve. The lower marker (dye molecule) peak elutes at ~28 s while the largest ladder peak (100 nt) elutes at 45 s. This assay delivers a very fast analysis time in which small RNA molecules can be measured and the results observed in real-time on the LabChip® GX Touch™ nucleic acid analyzer.

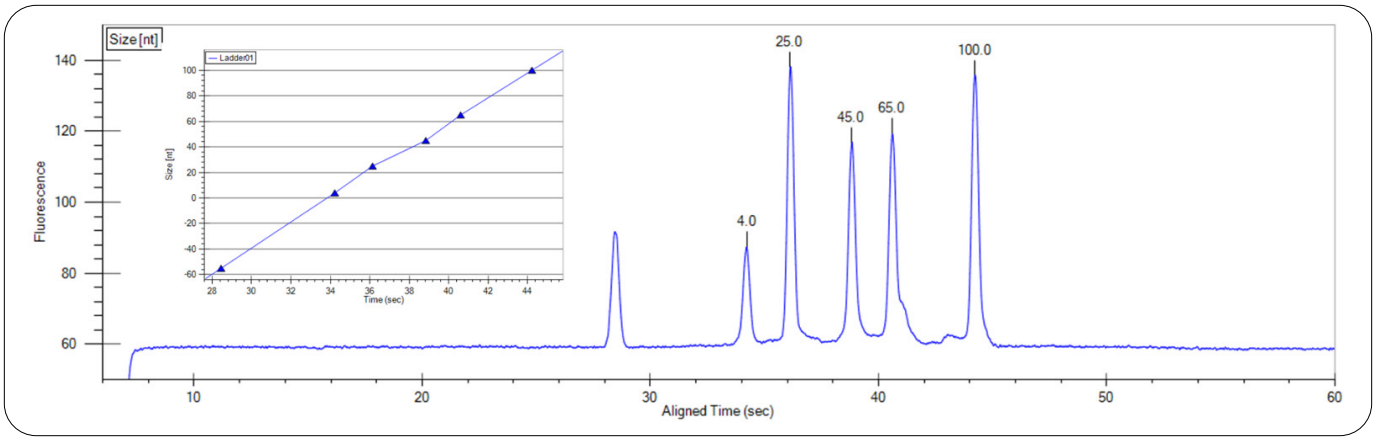


Figure 1: LabChip® Small RNA assay and ladder standard calibration curve. A ladder with sizing markers of 4, 25, 45, 65 and 100 nt are provided with the kit. The linearity of the sizing standard curve is shown in the inset.

To demonstrate assay performance, we performed analysis of 4 small RNA standard samples with varying sizes: 20 nt, 32 nt, 62 nt and 150 nt. For comparison purposes, they were also measured on another capillary electrophoresis instrument (reference instrument, Figure 2, purple bars). We then tested the sizing ability of the assay with the small RNA assay using 4 different chips on 2 LabChip® GX Touch™ nucleic acid analyzer instruments. The sizing results are shown in Figure 2. The LabChip® small RNA assay showed good concordance to the orthogonal method and was able to measure RNA molecules with sizes from 20 to 150 nt. The assay displayed a high degree of inter-run sizing precision, with inter-run CV of 5.5%, 8.2%, 4.1% and 1.1% for the 20, 32, 62 and 150 nt fragment peaks respectively.

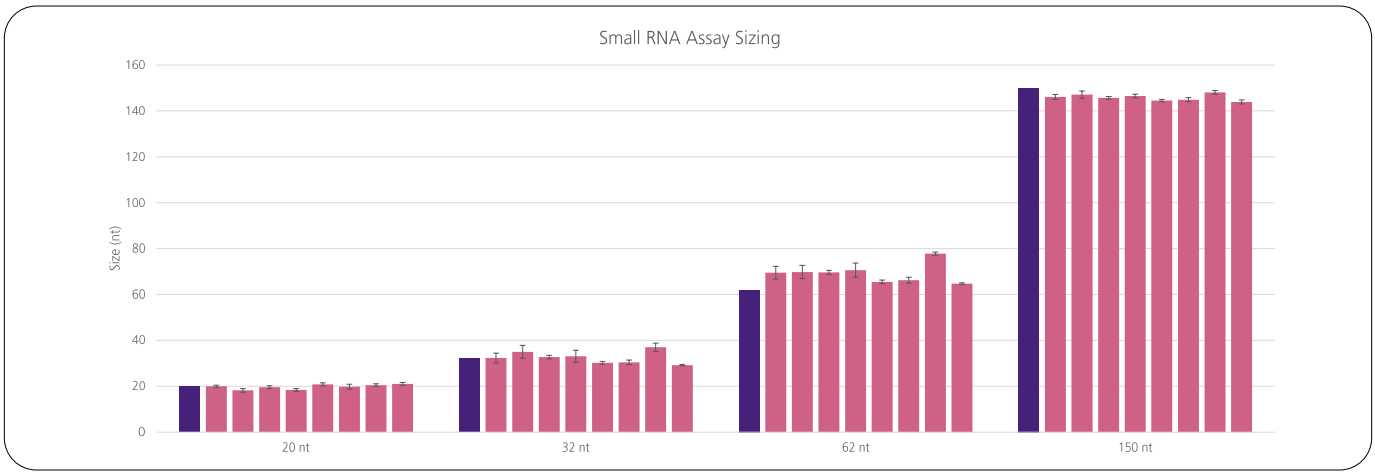


Figure 2: Small RNA sizing performance across multiple runs. Shown are the sizes observed for 8 runs of small RNA analytes compared to the size obtained using a reference instrument (purple bar). Each run (pink bar) sipped the RNA molecule 16 times. The error bar shown is the standard deviation of the 16 measurements.

The average sizing of the eight test runs and difference from the reference instrument are shown in Table 1.

Table 1: Comparison of average RNA size obtained on LabChip® Small RNA assay to reference instrument.

Fragment (nt)	Measured on reference instrument (nt)	Measured on LabChip® small RNA assay (Average of 512 sips, nt)	Error between orthogonal method
20	20	19.8 ± 1.1	-1.0%
30	32	31.9 ± 2.6	-0.3%
60	62	67.5 ± 2.8	8.9%
150	150	145.8 ± 1.6	-2.8%

The linearity of the assay was tested using the 20 nt and 150 nt fragments with on-plate concentration from ~2000 pg/µL to 50 pg/µL. An overlay of the dilution electropherograms is shown in Figure 3. Each titration curve gave an R2 value greater than 0.99. On-plate concentrations down to 50 pg/µL were detected representing the maximum sensitivity of the assay for samples run without dilution. For dilution of minimal amount of sample (2 µL), 1:5 using provided sample buffer, this represents an assay concentration range of 250 pg/µL to 1,000 pg/µL.

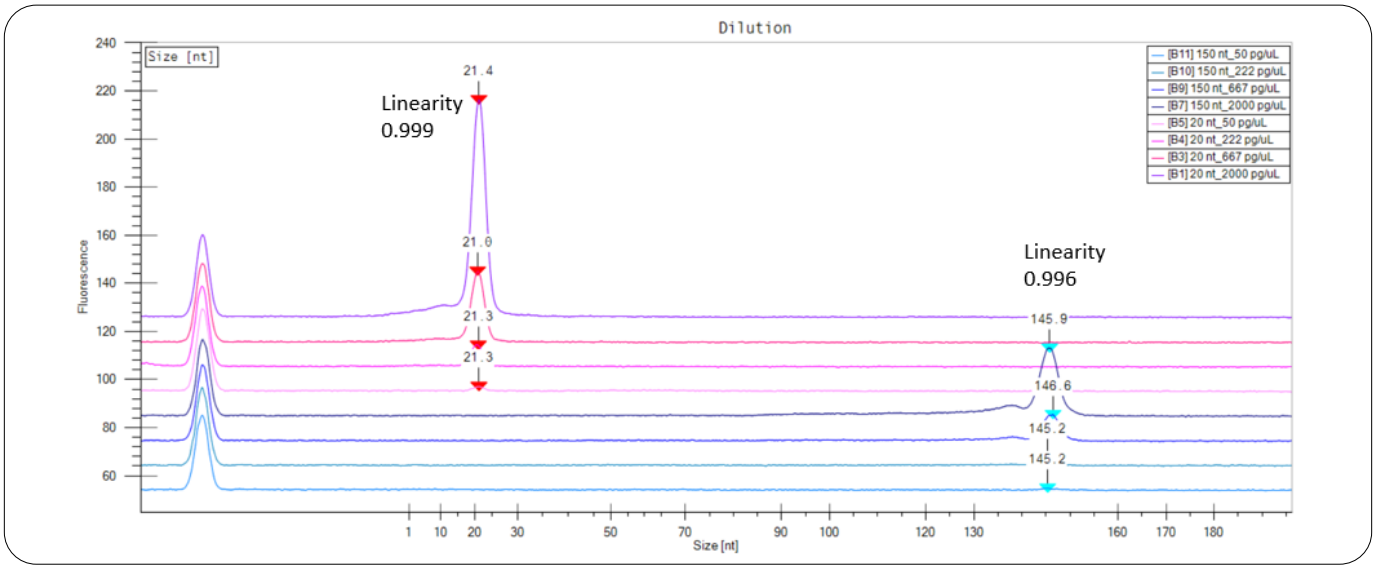


Figure 3: Small RNA electropherograms showing dilution of 20 and 150 nt samples from 2000 pg/uL to 50 pg/uL (on-plate concentration). The R2 value for the concentration dilution curve for each fragment is greater than 0.99.

The ability to measure small RNA (10-40 nt) directly in total RNA samples is also useful. This enables researchers to observe RNA extraction procedure efficacy and compare RNA content between different extraction methods and tissues. An example of this is shown in Figure 4, where Reference Human Brain RNA (P/N QS0611, Thermo Fisher Scientific) was analyzed using the LabChip® Small RNA assay at on-plate concentration of 6,800 pg/µL and 68,000 pg/µL.

RNA regions of interest may be quantified using smear analysis of the LabChip® GX Reviewer software. Shown below are smears corresponding to the microRNA (10-40 nt) and small RNA content (10-150 nt). In each trace, detection of the microRNA fraction from 10-40 nt was detected. More predominant small RNA peaks likely including transfer RNA molecules were also detected and the RNA size measured.

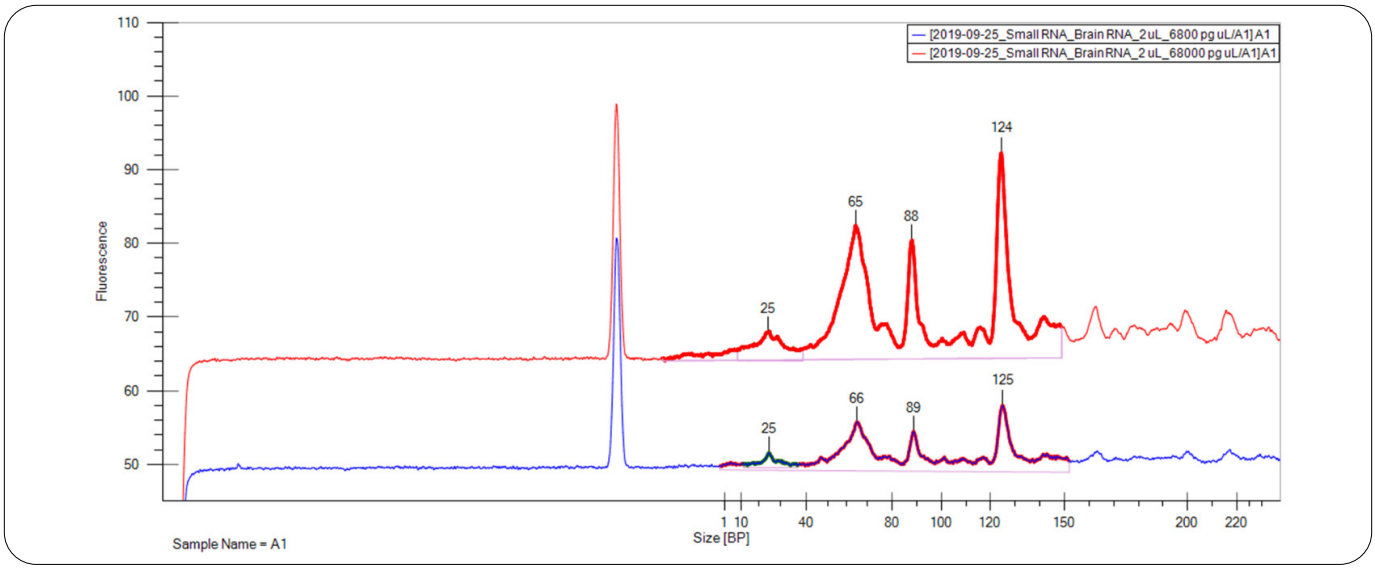


Figure 4: LabChip® Small RNA assay electropherogram of Human Brain Reference RNA at 6,800 pg/uL and 68,000 pg/uL in water. Detection of microRNA and transfer RNA peaks were detected.

Discussion

Together with LabChip® GX Touch™ nucleic acid analyzer, the new LabChip® Small RNA assay allows users to separate and measure small RNA molecules easily, quickly, and reliably. Minimum sample consumption, re-useable microfluidics chips, and automated workflow make the high-throughput quality control screening of small RNA molecules more productively and cost effectively in modern genomic research and biotherapeutic development process. The assay provides sizing, concentration, and percent purity for small RNA analytes. The new gel formulation achieves resolution of 5 nt for 20-100 nt range, and 10% size difference for 100-150 nt. This allows users to check for degradation and confirm correct RNA size prior to using synthetic RNA molecules in expensive protocols such as CRISPR-Cas 9 workflows. The assay may also be useful for development and manufacturing laboratories of RNA therapeutics, which are gaining importance in rare disease and immuno-oncology applications. RNA molecules in development or production may be rapidly measured, percent purity analyzed and checked for quality control. Users can resolve and analyze variety of RNA molecules ranging from 20-6,000 nt, calculate quality scores, and achieve high throughput analysis by combining both the LabChip® RNA Pico assay and LabChip® Small RNA assay.

Ordering information

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For technical support please contact revvity.com

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com