APPLICATION NOTE

Nucleic acid quantitation and analysis using the QuickDrop Spectrophotometer

Benefits

- **Minimal sample usage as little as 0.5 µL**
- **Accurate DNA Quantitation from 1.0 ng/µL to 2,500 ng/µL**
- **LCD touchscreen for stand-alone experimentation and data analysis**
- **Kinetic and wave scanning capabilities for cuvette reads**

Introduction

Spectrophotometry is a well-established technique used to quantitate and analyze biological substances. Of these substances, nucleic acids are one of the most routinely measured in life science laboratories. Determining the concentration and purity of these samples is extremely important for many downstream experiments such as PCR, qPCR, sequencing, and DNA microarrays.

Nucleic acids absorb ultraviolet light mainly at 260 nm, and their concentrations can be calculated by applying the Beer-Lambert law with their associated extinction coefficient and sample path length. First, ultraviolet light at 260 nm is directed into the sample, and as this light passes through the sample, a photodetector on the other side measures how much light was absorbed. By comparing the results to a reference (usually the sample diluent), one can quantitate a sample's nucleic acid concentration.

Sample purity is an important parameter in nucleotide quantitation. Although it is not the most accurate method for determining sample purity, the A260/A280 and A260/A230 ratios can be used as a rough estimate of protein and chemical contamination.

The SpectraMax® QuickDrop™ Spectrophotometer is a versatile ultraviolet-visible (UV-Vis) micro-volume spectrophotometer exceptional at analyzing nucleic acid samples. It contains a 0.5 mm micro-volume port, cuvette port, and built-in LCD touchscreen allowing the user to perform various experiments all on one system.

In this application note, we demonstrate how the QuickDrop spectrophotometer can both quantitatively (concentration) and qualitatively (sample purity) analyze nucleic acid samples with great accuracy and consistency.

Materials and methods

- QuickDrop UV-Vis Spectrophotometer (Molecular Devices cat. #QUICKDROP)
- UltraPure™ Calf Thymus DNA Solution (Thermo Fisher cat. #15633019)
- RNA Control 250 (Thermo Fisher cat. #AM7155)
- 10 mm Far UV Quartz Cuvettes (Starna Cells cat. #9-Q-10)

Sample carryover

Sample carryover was assessed by alternating measurements on the micro-volume port with calf-thymus DNA and ultra-pure water. Ultra-pure water was used as a reference. The micro-volume port was wiped with a lint-free wipe after each read.

Standard curve linearity

A two-fold dilution series starting from 2500 ng/µL of double-stranded DNA (dsDNA) was prepared using ultra-pure water. Ultra-pure water was used as a blank, and each sample concentration was read three times on the 0.5-mm micro-volume port. Using the pre-programmed DNA quantification method, absorbance was measured over wavelengths ranging from 230 nm to 320 nm. The DNA quantification method automatically calculated the dsDNA

concentration based on the equation shown below where A260 and A320 refer to the absorbance measured at 260 nm and 320 nm respectively.

Concentration in µg/mL =
(A_{260}–A_{320}) x dilution factor x 50 µg/mL

The QuickDrop automatically performs these calculations and reports the concentration to users. Afterwards, data were graphed using SoftMax® Pro Software and the SoftMax Pro Import Feature. A log-log curve fit was applied to demonstrate the linearity of the standard curve.

Sample volume comparison

Calf thymus DNA was prepared by diluting stock calf thymus DNA with ultra-pure water. 0.5 µL, 1.0 µL and 2.0 µL of samples were tested on the micro-volume port (n = 5). Ultra-pure water was used as a reference. Reference volumes were similar to the sample volumes tested.

RNA validation

ThermoFisher's RNA Control 250 was used to compare the QuickDrop's spectrophotometer performance to that of the NanoDrop™ Spectrophotometer. The storage solution provided with the kit was used as the reference. Both the reference sample and the RNA Control were assayed on the micro-volume port. Results were compared to the specifications provided in the RNA control kit.

Contaminant detection

Sample DNA was contaminated with a phenol solution resulting in a final concentration of 50 µM phenol in the solution. Contaminated sample and control were then analyzed in the cuvette port using the "Wavescan" feature. This feature incrementally checks the sample's absorbance over a range of wavelengths. Results were graphed using Microsoft® Excel.

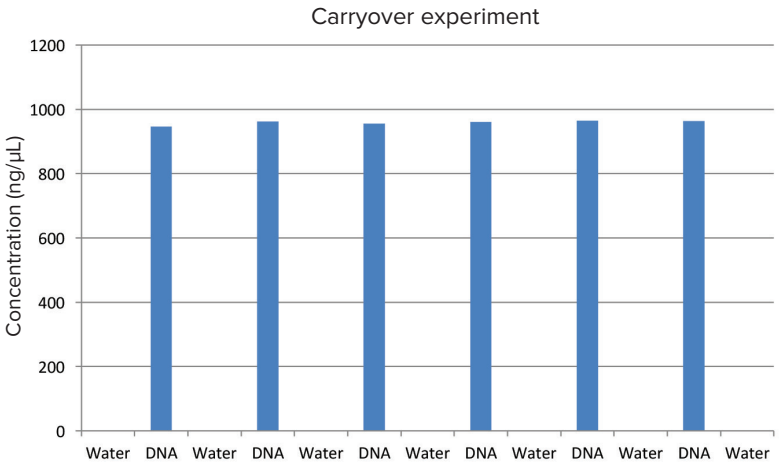


Figure 1. Sample carryover. Alternating measurements of calf thymus DNA and water were performed on the micro-volume port. Data were graphed on Excel. The data comprising this graph are shown in Table 1.

Sample type	Concentration (ng/uL)	A230	A260	A280	A320	A260/ A230	A260/ A280
Water	0.175	-0.001	-0.001	-0.002	-0.001	1	-0.212
DNA	946.344	0.396	0.947	0.52	0.001	2.394	1.822
Water	0	-0.026	-0.015	-0.015	-0.011	0.266	1
DNA	962.532	0.394	0.958	0.524	-0.005	2.415	1.821
Water	0	-0.027	-0.016	-0.015	-0.011	0.309	1.255
DNA	955.525	0.392	0.952	0.522	-0.004	2.416	1.818
Water	0	-0.026	-0.016	-0.015	-0.011	0.322	1.267
DNA	961.479	0.396	0.958	0.525	-0.003	2.407	1.819
Water	0	-0.024	-0.013	-0.013	-0.009	0.251	1
DNA	964.822	0.393	0.96	0.525	-0.005	2.425	1.821
Water	1.565	-0.006	0.001	0.001	-0.001	-0.288	1
DNA	963.753	0.394	0.958	0.524	-0.006	2.411	1.819
Water	0	-0.016	-0.006	-0.005	-0.002	0.288	1.328

Table 1: Data comprising the "Sample carryover" experiment in Figure 1.

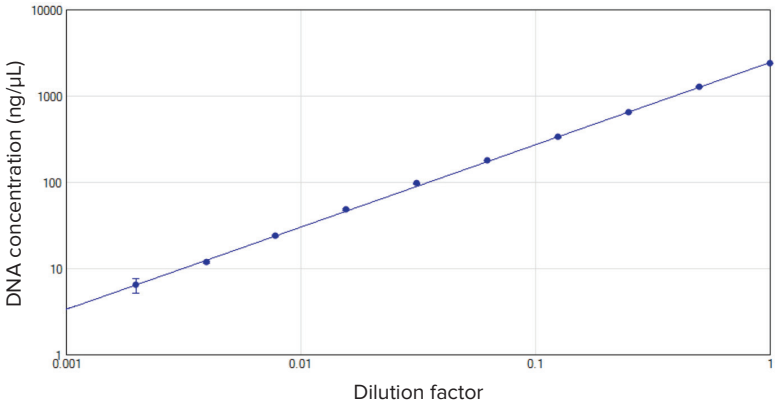


Figure 2. Calculated DNA concentration vs. dilution factor. A 2-fold dilution series of calf thymus DNA starting from 2500 ng/µL shows the relationship between the dilution factor and the DNA concentration reported by the QuickDrop. The curve demonstrated excellent linearity with a r^2 value of 1.000.

Results

Sample carryover

Sample carryover experiments were tested on the QuickDrop's micro-volume port. Results in Figure 1 and Table 1 demonstrated that with a simple wipe of a lint-free wipe, there was no significant sample carryover in the subsequent reads.

Standard curve linearity

In Figure 2, the QuickDrop demonstrated a linear relationship between the DNA concentration and the dilution factor. From this experiment, we determined that the 1 ng/µL DNA was the lowest measureable concentration for this QuickDrop.

Sample volume comparison

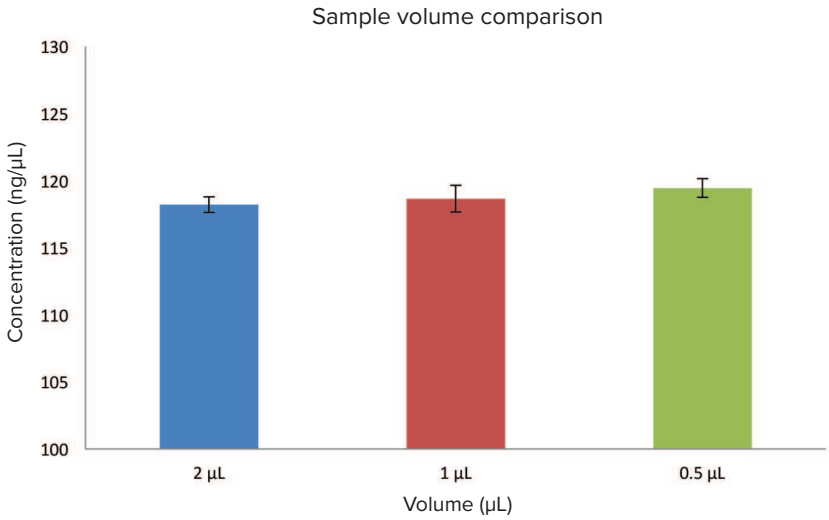
Three different volumes of sample DNA were assayed on the QuickDrop's micro-volume port. The calculated sample DNA concentration was consistent despite the volume difference (Figure 3). For best results, we recommend using a 2-µL volume as it was the easiest to pipette.

RNA validation

An RNA 250 control was measured to demonstrate the QuickDrop's ability to measure RNA. The measured concentration was within the accepted limits provided by the control kit (250 ± 5 ng/µL).

Contaminant detection

Using the Wavescan function, contaminants can be identified in samples due to the increase in absorbance at 230 nm compared to a control (Figure 5). This function can be applied to other types of contaminants found in the DNA purification process such as guanidine thiocyanate. The effective wavelength range of the QuickDrop extends from 190 nm to 1100 nm allowing for a comprehensive chemical analysis.



	Average	Standard deviation	%CV
2 µL	118.23	0.58	0.49
1 µL	118.66	0.99	0.83
0.5 µL	119.46	0.69	0.57

Figure 3. Volume reproducibility. 2 µL, 1 µL, and 0.5 µL volumes of calf-thymus DNA were read on the micro-volume port. Different volumes demonstrated very similar calculated concentrations (n = 5). Standard Deviation and %CV were very similar for all sample volumes.

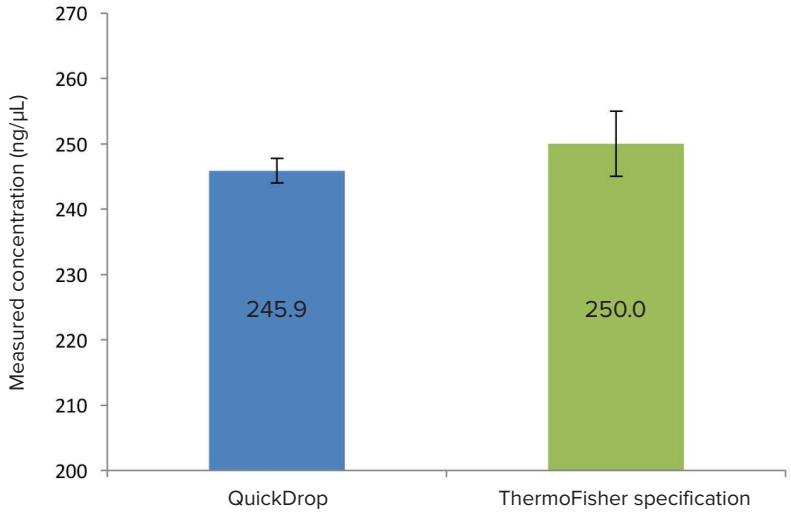


Figure 4. RNA validation experiment. 250 ng/µL RNA Control was measured on the QuickDrop to compare results to ThermoFisher's NanoDrop validation results (n = 4). The measured RNA concentration (245.9 ± 1.9 ng/µL) falls within ThermoFisher's specified range (250.0 ± 5.0 ng/µL).

Conclusion

The QuickDrop's built-in cuvette port and micro-volume sample port allow for both sensitive quantitation and analysis of nucleic acid samples. As shown above, as little as 0.5 µL of sample can be read on the micro-volume port with great consistency. Also, it has a wide detection range (1 ng/µL to 2500 ng/µL) and low sample carryover.

Finally, its built-in LCD touchscreen provides researchers with important information such as sample concentration and purity (Figure 6). All these features, along with its small footprint, make the QuickDrop Spectrophotometer an excellent choice for nucleic acid quantitation and analysis in any lab environment.

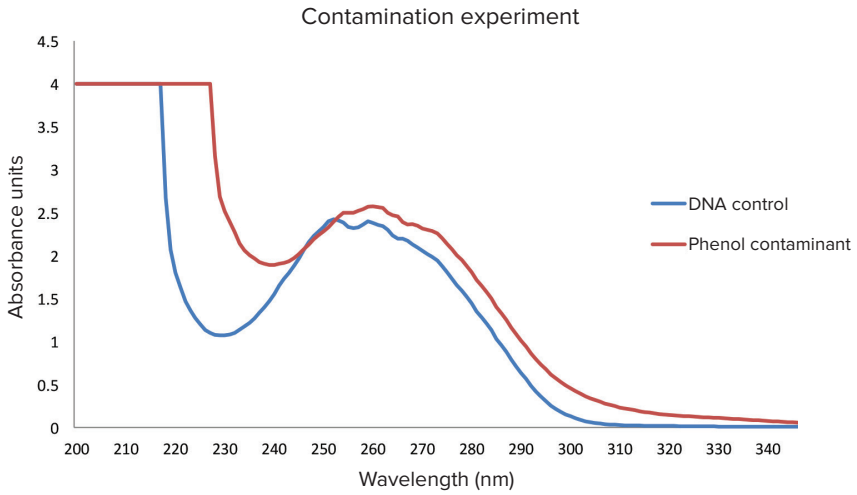


Figure 5. Contaminant experiment. Phenol-contaminated samples and an uncontaminated control were assayed using the Wavescan function. Wavescan traces were graphed and overlaid using Microsoft Excel. There is a definitive increase in absorbance at the 230 nm mark.

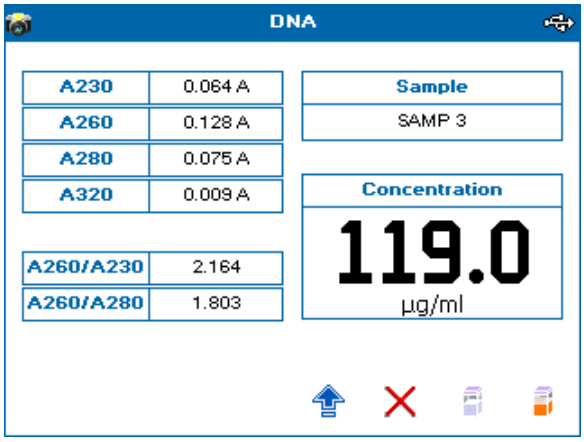


Figure 6. Example of QuickDrop's data display. Relevant parameters are calculated and reported on the QuickDrop's touchscreen.

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