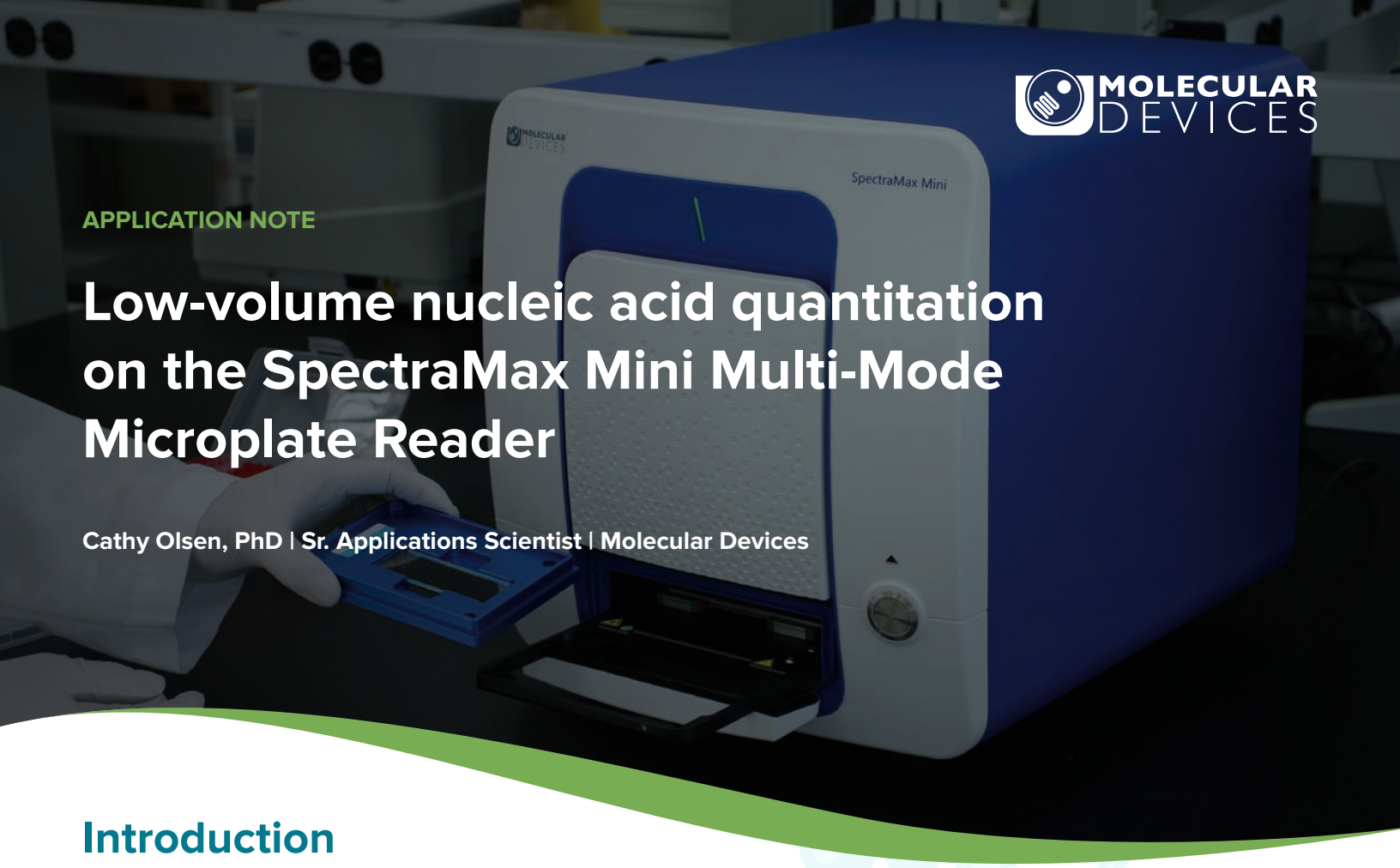

APPLICATION NOTE

Low-volume nucleic acid quantitation on the SpectraMax Mini Multi-Mode Microplate Reader

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Introduction

Nucleic acid quantitation is an essential assay that is part of many workflows in genetics, molecular, and cellular biology. Various methods have been developed to quantitate nucleic acids, but the most commonly used technique is still ultra-violet (UV) spectrophotometry. The basis of spectrophotometry is that every molecule absorbs or transmits light over a certain wavelength range, and concentrations can be calculated by using the Beer-Lambert law (equation below) with preceding knowledge of the sample's molar extinction coefficient and the measurement pathlength.

$A = \varepsilon cL$

The Beer Lambert law states that absorbance (A) is equal to the measured sample's molar extinction coefficient (ε) multiplied by the concentration (c) and pathlength (L) used to measure the sample. Re-arranging the equation allows us to use the measured absorbance value to calculate concentration.

Nucleic acid quantitation is a very well-established technique and is one of the most widely used methods in life science laboratories. To calculate nucleic acid concentration, the absorbance of a sample is measured at 260 nm (auxiliary measurements may be taken at 230 nm and 280 nm wavelengths to help assess sample purity but are not necessary for determining the concentration). The absorbance measurement at 260 nm, along with the sample's extinction coefficient and pathlength are inserted into the equation above to arrive at the sample concentration.

Benefits

- Low-volume nucleic acid quantitation using as little as 2 µL of sample
- High-throughput measurement of 24 or 64 samples at a time
- Linear dynamic range of 3 decades for accurate quantitation without the need for dilution

Measuring samples one by one in cuvettes poses the problems of low throughput and large sample volume requirements. The SpectraDrop™ Micro-Volume Microplate allows users to read up to 64 samples per plate on SpectraMax® Microplate Readers, with sample volumes as low as 2 µL. The SpectraDrop Microplate incorporates a specially designed adapter and a slide pair whose optical clarity allows measurements in absorbance and fluorescence modes to meet users' application needs (Figure 1). The SpectraDrop Microplate's slide design eliminates the need for calibration and gives consistent well-to-well reads with CVs below 5%. In combination with the SpectraMax Mini Multi-Mode Microplate Reader, it enables increased throughput and detection of DNA as low as 2 ng/µL using just a few microliters of sample.

Materials

- SpectraDrop Micro-Volume Microplate
- Double-stranded DNA
  (UltraPure™ Calf Thymus DNA Solution, 10 mg/mL,
  ThermoFisher Scientific cat. #15633019)
- TE buffer
- 8-channel pipettor capable of pipetting 2 or 4 µL
- SpectraMax® Mini Multi-Mode Microplate Reader

Methods and results

DNA standards of known concentrations ranging from 0.98 ng/µL to 1000 ng/µL were prepared in TE buffer and pipetted onto the wells of the 64-well SpectraDrop plate, along with TE buffer blanks, in sets of four replicates using an 8-channel pipettor. The volume per sample was 2 µL when using the SpectraDrop top slide with 0.5-mm spacers, or 4 µL when using the top slide with 1-mm spacers.

The SpectraDrop plate was read on the SpectraMax Mini reader, and data were analyzed and graphed with SoftMax® Pro Software.

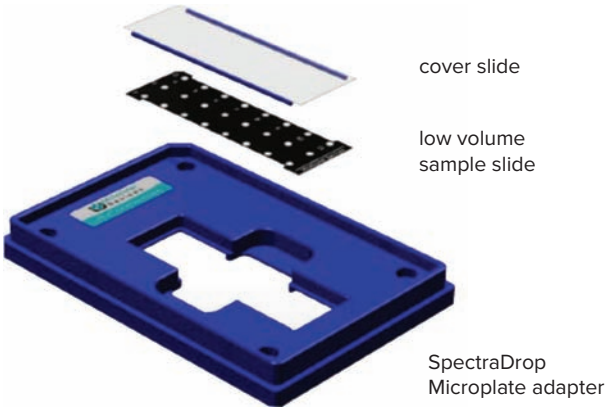

Figure 1. *SpectraDrop Micro-Volume Microplate configuration. The low-volume sample slide has a mask delineating spots or 'wells' that hold 24 or 64 samples. The cover slide has 0.5-mm or 1.0-mm spacers for use with 2-µL or 4-µL samples, respectively.*

Standards representing a three-decade range of DNA concentrations, from 0.98 ng/µL up to 1000 ng/µL, were linear when plotted using either a linear or log-log curve fit with SoftMax Pro Software, with r^2 values greater than 0.99 (Figure 2).

The lower limit of detection for this double-stranded DNA, based on a calculation of three times the standard deviation of the background, was less than 3 ng/µL when using 2-µL samples, and less than 2 ng/µL when using 4-µL samples.

Conclusion

The SpectraMax Mini Multi-Mode Microplate Reader is a compact and versatile microplate reader capable of quantitating samples in a variety of microplate formats. When combined with the SpectraDrop Micro-Volume Microplate, the reader can quantitate up to 64 samples with as little as 2 µL sample volume in a single read.

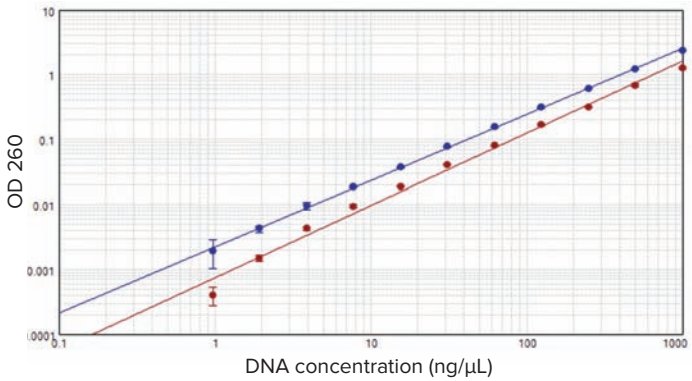

Figure 2. *DNA standard curves on the SpectraDrop plate, read on the SpectraMax Mini reader. Blue plot shows 2-µL samples; red plot shows 4-µL samples (r^2 values were 0.991 and 1.000, respectively).*

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