APPLICATION NOTE

Low-volume dsDNA quantitation using SpectraMax Quant dsDNA Assay Kits

Introduction

Double-stranded DNA (dsDNA) quantitation is an important precursor to many downstream molecular biology experiments such as qPCR, plasmid transfections, and next-generation sequencing. DNA quantitation is commonly accomplished using ultraviolet (UV) spectrophotometry, however, this method has several limitations. It cannot be used to quantitate DNA below 250 ng/mL, and it requires a relatively large sample volume. Results are also affected by contaminating substances such as RNA and protein. A fluorometric approach is a preferred method to determine dsDNA concentration due to its high sensitivity and specificity.

Molecular Devices SpectraMax® Quant family of dsDNA quantitation kits allows for very accurate quantitation of dsDNA in a linear range from 0.5 pg/µL to 200 ng/µL. Normally this fluorometric assay is performed in 96-well microplates. For valuable samples and higher-throughput requirements, the assays can be performed in 384-well microplates.

In this application note, we show the optimal settings and protocol for performing dsDNA quantitation in a 384-well format using SpectraMax Quant dsDNA Assay Kits. This method saves precious sample DNA and reduces the number of microplates used.

Materials and methods

- SpectraMax® Quant™ AccuBlue™ Pico dsDNA Assay Kit (Molecular Devices cat. # R8354)
- SpectraMax® Quant™ AccuClear™ Nano dsDNA Assay Kit (Molecular Devices cat. # R8356)
- SpectraMax® Quant™ AccuBlue™ HiRange dsDNA Assay Kit (Molecular Devices cat. # R8358)
- UltraPure™ Calf Thymus DNA (Thermo Fisher cat. # 15633019)
- Greiner 384-well solid black flat-bottom microplate (Greiner cat. # 781-076)
- Greiner 96-well solid black flat-bottom microplate (Greiner cat. # 655-076)
- SpectraMax® i3x Multi-Mode Microplate Reader

Benefits

- **Save precious sample DNA**
- **Increase throughput and save microplates**
- **Assay a wide range of concentrations (0.5 pg/µL to 200 ng/µL DNA)**

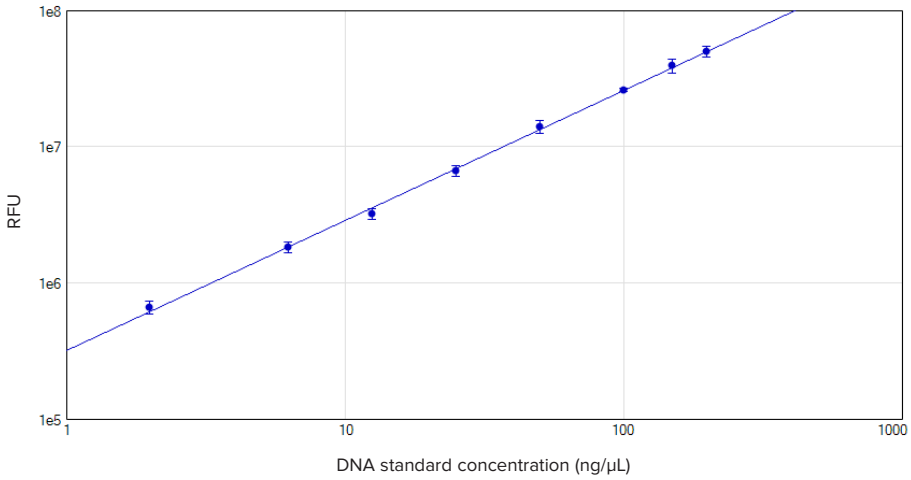

Figure 1. SpectraMax Quant AccuBlue HiRange standard curve run on a 384-well microplate. A log-log curve fit was applied. R^2 = 0.999. PMT gain was set to Auto, with 10 flashes/read.

Protocol

Working solution for each dsDNA quantitation assay kit was prepared according to the respective product inserts. 50 µL of working solution was pipetted into each well of a 384-well solid black microplate. 2.5 µL of DNA was added to each well and the microplate was shaken to ensure proper mixture. Afterwards, the microplate was spun for one minute at 1000 rpm. The microplate was then incubated in the dark for 10 minutes before being read on the SpectraMax i3x Multi-Mode Microplate Reader.

Unknown sample DNA was also assayed in both a 96-well microplate and a 384-well microplate and concentrations were calculated using preconfigured protocols in SoftMax® Pro Software.

Results

Standard curves for the SpectraMax Quant dsDNA Assay Kits on the 384-well microplates demonstrated R^2 values > 0.99 (Figures 1–3). The lower limits of detections (LLD) for the 384-well protocols are shown in Table 1. In Figure 4, the standard curves of a 96-well microplate and 384-well microplate were compared, and both curves demonstrated similar slopes. Unknown dsDNA sample concentrations were calculated using these curves and reported in Table 2.

Conclusion

Running the SpectraMax Quant dsDNA Assay Kits using a 384-well format demonstrated detection ranges comparable to the 96-well format with improving assay throughput. With the 384-well format, users can quantitate dsDNA using one-fourth the normal required amount of sample DNA.

SpectraMax Quant dsDNA Assay Kit	Calculated LLD
Pico	0.18 pg/µL
Nano	0.002 ng/µL
Hi-Range	0.200 ng/µL

Table 1. Calculated Lower Limit of Detection (LLD) for each of the dsDNA quantitation assay kits.

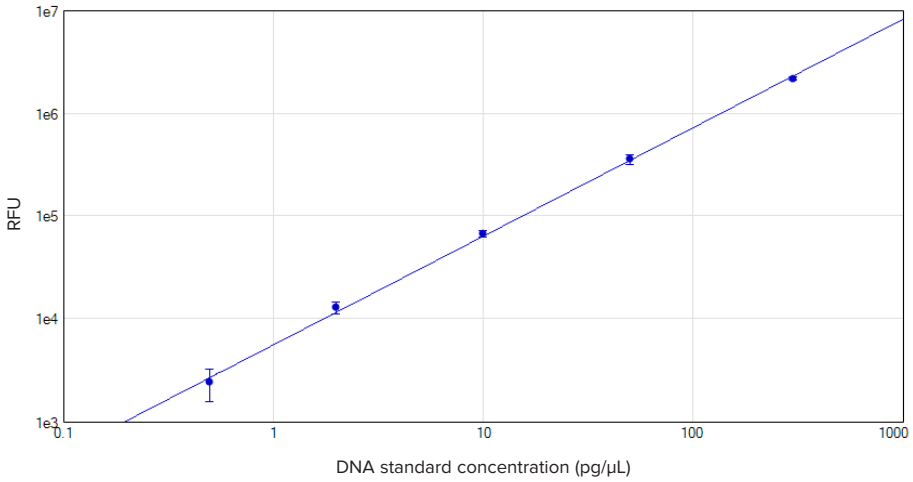

Figure 2. SpectraMax Quant AccuBlue Pico dsDNA standard curve run on a 384-well microplate. A log-log curve fit was applied. R^2 = 0.999. PMT gain was set to Auto, with 10 flashes/read.

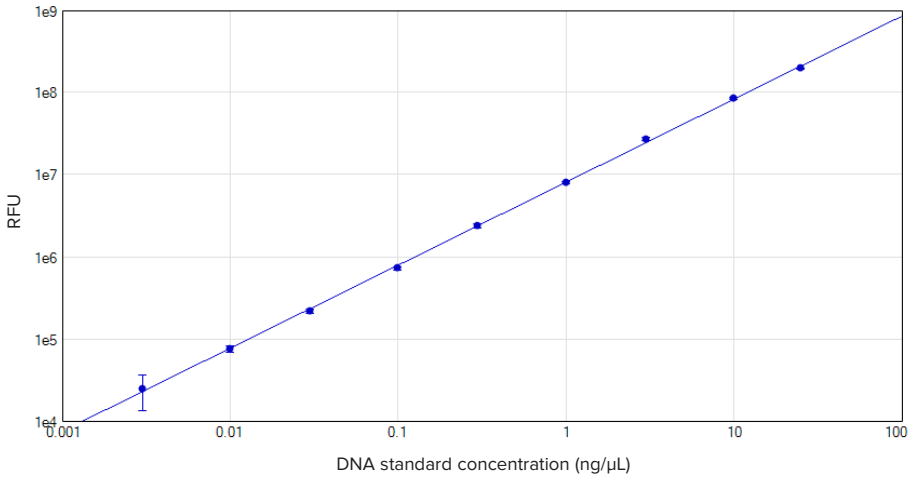

Figure 3. SpectraMax Quant AccuClear Nano standard curve run on a 384-well microplate. A log-log curve fit was applied. R^2 =1.000. PMT gain was set to Auto, with 10 flashes/read.

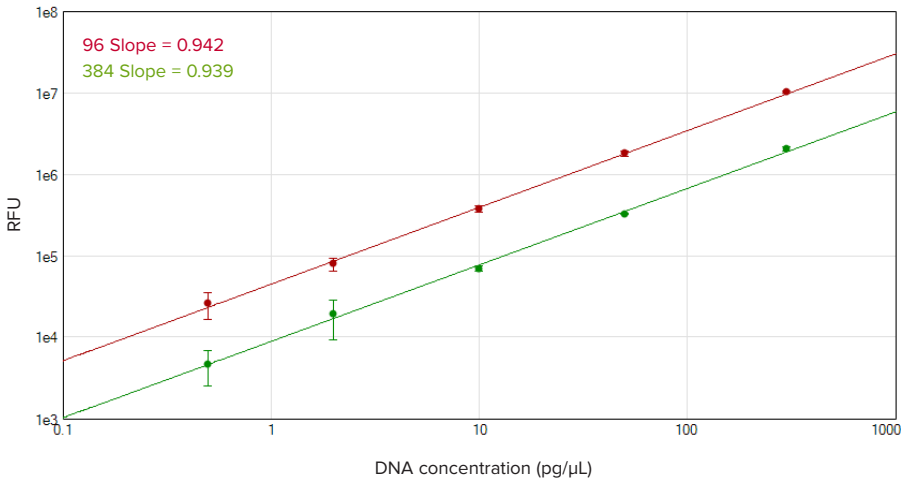

Figure 4. Comparison between 96- and 384-well methods with unknowns. A log-log curve fit was applied to both data sets. The 96-well standard curve is shown in red and the 384-well standard curve is shown in green. Both standard curves demonstrated R^2 values > 0.998.

	Actual concentration	96-well format	384-well format
Unknown 1	3 pg/µL	2.92 pg/µL	2.78 pg/µL
Unknown 2	30 pg/µL	29.41 pg/µL	27.59 pg/µL

Table 2. Unknown dsDNA quantitation. Unknown dsDNA concentrations were quantified and compared using the SpectraMax Quant AccuBlue Pico kit in a 96-well or 384-well microplate.

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