APPLICATION NOTE

Quantitation of dsDNA samples using the SpectraMax Quant dsDNA Assay Kits

Introduction

DNA is present in nearly all living cells and contains the molecular instructions for life. Reliable and accurate quantitation of DNA is an essential step in molecular biology research including next-generation sequencing, genotyping, etc. Among different methods available for quantifying double-stranded DNA (dsDNA), fluorometric assays performed with microplate readers have become increasingly popular as they provide several key advantages including significantly increased sensitivity, high selectivity for dsDNA over single-stranded DNA (ssDNA) or RNA, and improved tolerance for contaminants typically found in the DNA purification process. The microplate format also enables higher throughput and lower sample volume.

As different types of test samples may contain different concentrations of DNA, selection of an appropriate fluorescent reagent with the required sensitivity and detection range is important. In this application note, we demonstrate the use of SpectraMax® Quant™ dsDNA Assay Kits for precise quantitation of DNA samples ranging from 5 picogram (pg) to 2000 nanogram (ng) per well. Data are acquired using a SpectraMax® fluorescence microplate reader and rapidly analyzed with a preconfigured protocol in SoftMax® Pro Software.

Materials and methods

SpectraMax Quant dsDNA Assay Kits

The SpectraMax Quant dsDNA Assay Kits consist of three products, each designed for a specific range of DNA concentrations and optimized for use with fluorescence microplate readers such as the SpectraMax microplate reader (Table 1). An enhancer is provided in the kit where applicable to further reduce background fluorescence and to increase the dynamic range of the assay. Details on product configuration are available at www.moleculardevices.com.

SpectraMax i3x microplate reader and SoftMax Pro Software protocol

For each assay kit, a preconfigured protocol is included in SoftMax Pro Software to facilitate the acquisition and analysis of data for a simplified workflow. The protocols are also available on the SoftMax Pro Software protocol sharing web site (www.softmaxpro.org). To find the preconfigured protocols, launch the software and select the “Protocol” tab. Open the Protocol Manager and select the “Protocol Library”. Scroll down to the folder “Nucleic Acids” and open the preconfigured protocol that matches the DNA quantitation kit you are using by clicking on its name.

Benefits

- **Highly selective for dsDNA over single-stranded DNA or RNA**
- **Three different kits cover a wide range of concentrations**
- **Optimized for SpectraMax microplate readers**
- **Simplified data acquisition and analysis with preconfigured protocol in SoftMax Pro Software**

Assay kit	Linear detection range (per well)	Fluorescence reader setting (Excitation/Emission)
SpectraMax® Quant™ AccuBlue™ Pico dsDNA Assay Kit	0.005 ng – 3 ng	468 nm/507 nm
SpectraMax® Quant™ AccuClear™ Nano dsDNA Assay Kit	0.03 ng – 250 ng	468 nm/507 nm
SpectraMax® Quant™ AccuBlue™ HiRange dsDNA Assay Kit	2 ng – 2,000 ng	350 nm/460 nm

Table 1. SpectraMax Quant dsDNA Assay Kits.

Experimental protocol

Components of the kits were allowed to reach room temperature. A standard curve for each of the SpectraMax Quant dsDNA Assay Kits was prepared using the standards provided with each kit. The general protocol for preparing the working solution for each kit is similar; however, buffers, dyes, and enhancers are different. See Table 2 for more information about the buffers and working solution concentrations. 10 µL of dsDNA standard sample was pipetted into wells of a black 96-well plate (Greiner cat. #655076), followed by 200 µL of working solution. One plate was made for each standard curve. In addition, the same concentrations of ssDNA and RNA were plated for each kit to compare dye specificity to dsDNA. Sample plates were mixed by shaking, covered, for five minutes; then fluorescent signal was detected on the microplate reader. Data were acquired and automatically analyzed using the preconfigured protocol specific to each kit in SoftMax Pro Software. The workflow is shown in Figure 1. Detailed instructions can be found in the respective product inserts. When testing unknown samples, concentrations are interpolated from a standard curve and reported in the "Samples" group table in the "Mean Result" column.

Assay kit	Buffer	Dye	Enhancer
SpectraMax® Quant™ AccuBlue™ Pico dsDNA Assay Kit	Dilute 1:20 in deionized water	Dilute AccuBlue Pico dye 1:400 in AccuBlue Pico Buffer	Dilute 1:100 in same tube with dye
SpectraMax® Quant™ AccuClear™ Nano dsDNA Assay Kit	Dilute 1:20 in deionized water	Dilute AccuClear Nano dye 1:100 in AccuClear Nano Buffer	NA
SpectraMax® Quant™ AccuBlue™ HiRange dsDNA Assay Kit	Do not dilute	Dilute AccuBlue HiRange dye 1:100 in AccuBlue HiRange Buffer	Dilute 1:100 in same tube with dye

Table 2. SpectraMax Quant dsDNA Assay Kit working solution components.

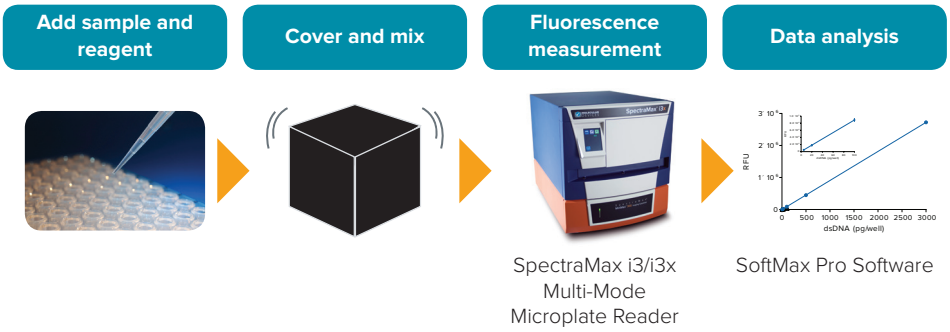

Figure 1. SpectraMax Quant dsDNA Quantitation Assay Kits workflow.

Results

SoftMax Pro Software automatically analyzes and plots the data after the read. By default, the standard curve is plotted with a linear curve-fit, but a log-log curve fit is also available in the dropdown Curve Fit menu of the Graph section. Standard curves for each of the three SpectraMax Quant dsDNA Assay Kits are shown in Figure 2. Each one had an R^2 value of 0.999. The fluorescent signal from the dsDNA in the standard curves was compared to the fluorescence from equal concentrations of single-stranded DNA and RNA. All three kits are selective for dsDNA. Results are shown in Figure 3.

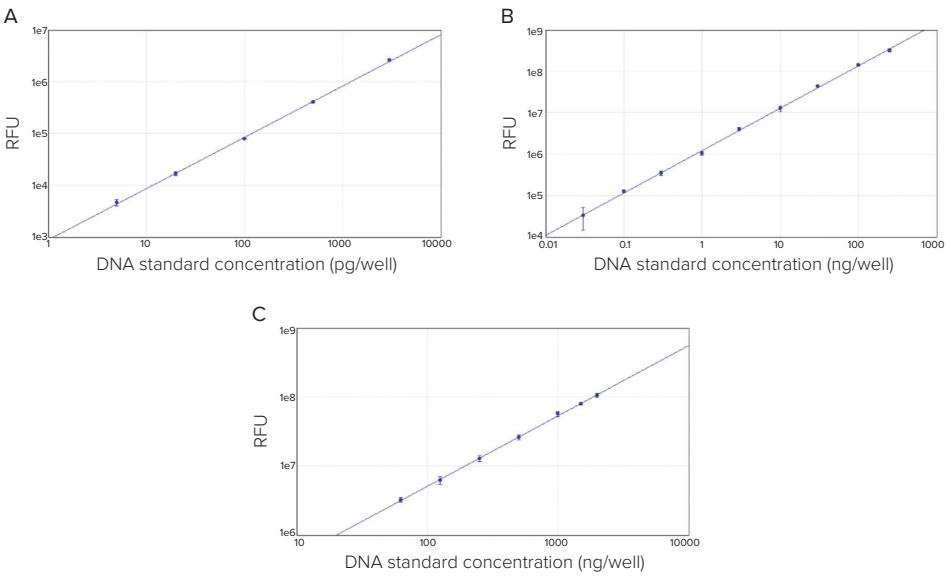

Figure 2. Standard curve comparisons. Standard curves for the SpectraMax Quant dsDNA Assay Kits are shown here. **(A)** SpectraMax Quant AccuBlue Pico assay; **(B)** SpectraMax Quant AccuClear Nano assay; **(C)** SpectraMax Quant AccuBlue HiRange assay. R^2 = 0.999 for each curve.

Conclusion

The SpectraMax Quant dsDNA Assay Kits accurately quantify a broad range of concentrations of dsDNA with high selectivity. When used in conjunction with the SpectraMax readers and preconfigured protocols in SoftMax Pro Software, these kits enable a simplified workflow and accurate results for determining the concentration of unknown dsDNA samples.

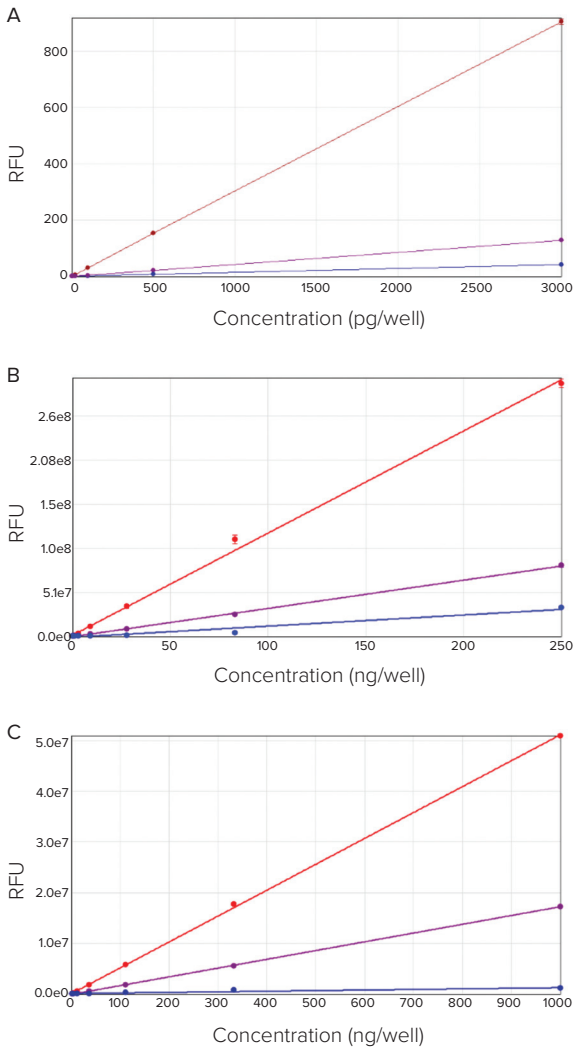

Figure 3. Selectivity of the SpectraMax Quant dsDNA Assay Kits. Fluorescent dye favorably binds dsDNA (Red) over ssDNA (Purple) and RNA (Blue). **(A)** AccuBlue Pico kit; **(B)** AccuClear Nano kit; **(C)** AccuBlue HiRange kit.

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