

APPLICATION NOTE

Quant-iT PicoGreen dsDNA assay with SpectraMax Microplate Readers

Introduction

Double-stranded DNA is typically quantitated in microplate readers by measuring the absorbance of the DNA solution at 260 nm. However, this method is only able to measure down to about 250 ng/mL on a typical absorbance microplate reader. For biological applications involving small samples, such as next-generation sequencing and quantitation of DNA amplification products, more sensitive methods are needed. The Quant-iT PicoGreen dsDNA Assay from Life Technologies is more specific for DNA and is about 1000 times more sensitive than traditional absorbance methods. The dynamic range of this assay in microplate format, as stated in the product information, is from 250 pg/mL to 1000 ng/mL with a single dye concentration.¹ This application note demonstrates that with Molecular Devices' SpectraMax[®] Fluorescence Microplate Readers and the Quant-iT PicoGreen assay, users can reliably measure down to at least 100 pg/mL of double-stranded DNA.

To maximize sensitivity of the assay, it is necessary to use optimal excitation and emission wavelengths. Unlike filter-based plate readers, the dual monochromators in SpectraMax Microplate Readers allow the selection of any wavelength within the reader's stated range. It is important to determine the excitation and emission wavelengths that provide the best dynamic range for the assay, since these may differ somewhat from those used with filter-based plate readers. Optimal excitation

and emission wavelengths for SpectraMax M5 fluorescence microplate readers are as follows: excitation at 490 nm and emission at 525 nm, with 515-nm emission cutoff filter (note that these are different from the wavelengths recommended for filter-based readers in the Quant-iT PicoGreen product insert). A preconfigured protocol is available in SoftMax[®] Pro software, which is used to acquire, display, and analyze data from SpectraMax plate readers.

Materials

- Quant-iT PicoGreen dsDNA Assay Kit, including lambda DNA standard (Life Technologies cat. #P7589 or P11496)
- Black 96-well plate (Greiner Bio-One, cat. #655096)
- Brown or amber (light-blocking) microcentrifuge tubes
- Molecular Devices microplate reader with fluorescence detection mode:
 - SpectraMax[®] M3/M4/M5/M5e (cat. #M3 or #M4 or #M5 or M5E)
 - SpectraMax[®] i3x (cat. #i3x)
 - SpectraMax[®] M2/M2e (cat. #M2 or M2E)
 - Gemini XPS/EM (cat. #XPS or EM)
 - FlexStation[®] 3 (cat. #FLEX3)
 - FilterMax F3/F5 (cat. #F3 or F5)
 - SpectraMax[®] Paradigm (cat. #PARADIGM)
- SoftMax[®] Pro Software (Molecular Devices)

Benefits

- **Sensitive fluorescent quantitation of DNA down to 63 pg/mL**
- **Linear dynamic range spanning four orders of magnitude**
- **Easy analysis of results with preconfigured protocol in SoftMax Pro Software**

Methods

Instrument setup

- Turn on the microplate reader.
- Launch SoftMax Pro Software and open the PicoGreen Fluorescence protocol from the Protocols dropdown menu. Optimal settings for the assay are included in the protocol (see Table 1). Select Wells to Read and Assay Plate Type by clicking on “Settings” and locating the options on the left side of the screen.
- Click the Template button to open a window where you can assign wells of the microplate to pre-set template groups. Use the drop-down menu to select the appropriate template group. There are pre-configured template groups in the PicoGreen Fluorescence protocol including Standards, Unknowns, and Unknowns_NoDiln (for undiluted samples). Assigning wells to preset template groups populates group tables in the protocol with the corresponding data that is acquired when the microplate is read.

Prepare the assay

The method for this assay follows the instructions in the product information sheet for QuantiT PicoGreen dsDNA Reagent and Kits, except the assay volume is proportionately reduced from 2.0 mL to 200 μ L to fit a 96-well microplate format.

- Prepare 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) by diluting the concentrated buffer from the kit 20-fold with distilled DNase-free water.
- Prepare an aqueous working solution of QuantiT PicoGreen reagent by making a 200-fold dilution of the concentrated DMSO solution in TE buffer (prepared above). Preparation of the solution in a plastic container, rather than glass, is recommended, as the reagent may adsorb to glass surfaces. Protect the solution from light by using amber or brown tubes, or by covering with foil. This solution should be used within a few hours of its preparation.
- DNA standard curve: Prepare a 2 μ g/mL stock solution of dsDNA in TE. The lambda DNA standard provided with the kit can be diluted 50-fold in TE to make the 2 μ g/mL solution.

Parameter	Setting
Read mode	Fluorescence (Top Read)
Wavelengths	Excitation 490 nm, Emission 525 nm, Cutoff 515 nm
Sensitivity	Readings: 6, PMT: Auto
AutoCalibrate	On
Assay Plate Type	96-Well Standard Opaque

Table 1. Instrument settings for the QuantiT PicoGreen Assay on SpectraMax Fluorescence Plate Readers.

Volume (μ L) of TE	Volume (μ L) of 2 μ g/mL DNA Stock	Volume (μ L) of Diluted QuantiT PicoGreen Reagent	Final DNA Concentration in QuantiT PicoGreen Assay
0	1000	1000	1 μ g/mL
900	100	1000	100 ng/mL
990	10	1000	10 ng/mL
999	1	1000	1 ng/mL
1000	0	1000	Blank

Table 2. Preparation of high-range DNA standard curve.

Volume (μ L) of TE	Volume (μ L) of 50 ng/mL DNA Stock	Volume (μ L) of Diluted QuantiT PicoGreen Reagent	Final DNA Concentration in QuantiT PicoGreen Assay
0	1000	1000	25 ng/mL
900	100	1000	2.5 ng/mL
990	10	1000	250 pg/mL
999	1	1000	25 pg/mL
1000	0	1000	Blank

Table 3. Preparation of low-range DNA standard curve.

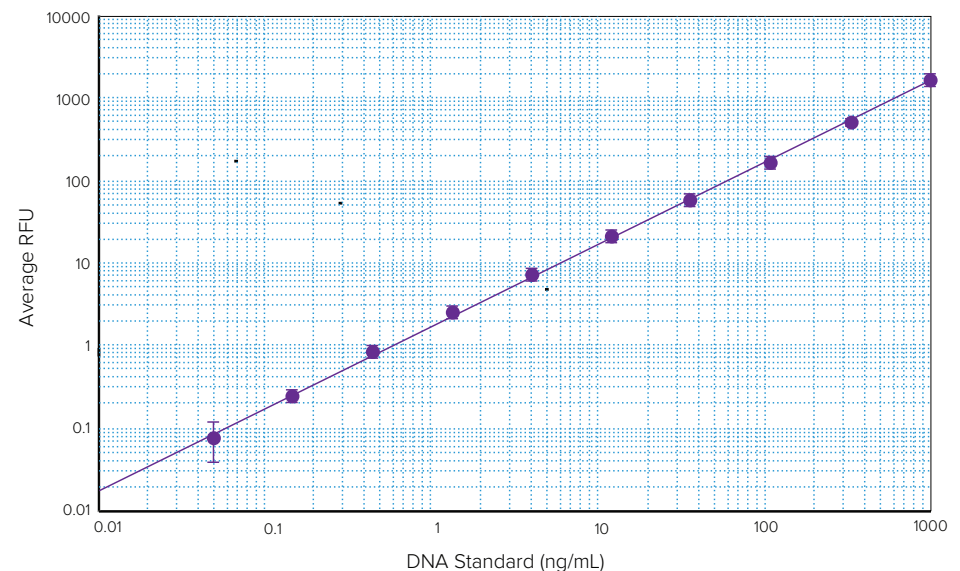


Figure 1. DNA standard curve. DNA standards ranging from 1000 ng/mL to 50 pg/mL were assayed using the QuantiT PicoGreen dsDNA Assay and SpectraMax M5 microplate reader. The standard curve was plotted using a log-log curve fit in the SoftMax Pro Software ($r^2 = 1.000$).

Note: in some cases it may be preferable to make the standard curve using DNA similar to the type being assayed.

- A high-range standard curve may be prepared from 1 ng/mL to 1 µg/mL, or a low-range standard curve may be prepared from 25 pg/mL to 25 ng/mL. For the high-range curve, follow the dilution scheme shown in Table 2; for the low-range curve, dilute the 2 µg/mL solution 40-fold to yield a 50 ng/mL solution, and refer to the dilution scheme in Table 3. Note: For this application note, a series of standards ranging from 1 µg/mL to 50 pg/mL were used.
- Pipet standards into a solid black 96-well microplate at 100 µL per well, preferably in triplicate. Be sure to include a set of buffer blank wells containing TE only (no DNA).
- Add 100 µL of the aqueous working solution of Quant-iT PicoGreen reagent to each well. Mix well by trituration or plate shaker and incubate for 2 to 5 minutes at room temperature, protected from light.

Read the microplate

- Make sure the purple plate adapter (if required for your plate reader) is in the microplate reader drawer. Place the microplate in the drawer.
- Click the Read button in the SoftMax Pro Software. The instrument will read the plate and the relative fluorescence units will be displayed in the Plate section of the protocol.

Analyze the data

- After the microplate has been read, the relative fluorescence units (RFUs) will be displayed in the Plate section. The data will be analyzed in the Group Tables that were created when the template was set up. For an example of representative data from a Group Table, see Table 4 in the Results section, following.
- Standards assigned in the Template (and thus displayed in the Standards group table) will be automatically plotted in the Standard Curve section of the protocol. A linear curve fit is applied by default, but a log-log fit may be used when plotting a standard curve over a wide dynamic range. Curve fits are chosen from the drop-down Curve Fit menu in the graph section.

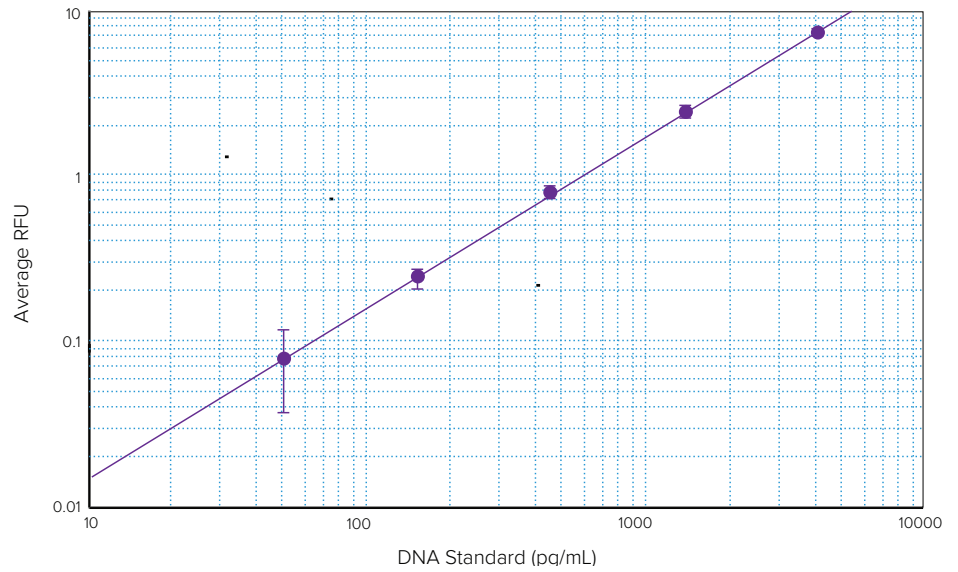


Figure 2. Lower end of DNA standard curve. Plot of the lower end of the DNA standard curve, ranging from 4.1 ng/mL to 50 pg/mL ($r^2 = 1.000$).

DNA Concentration (ng/mL)	Average RFU	Std Dev	%CV
1000.00	1608.421	19.679	1.2
333.33	501.927	4.323	0.9
111.11	163.556	2.417	1.5
37.04	56.884	0.753	1.3
12.35	20.777	0.307	1.5
4.11	7.148	0.057	0.8
1.37	2.433	0.065	2.7
0.46	0.8	0.047	5.9
0.15	0.237	0.031	12.9
0.05	0.076	0.039	50.9

Table 4. DNA standards.

Results

DNA standards ranging from 1 µg/mL to 50 pg/mL were detected using the Quant-iT PicoGreen dsDNA Assay and SpectraMax® M5 microplate reader (other fluorescence plate readers from Molecular Devices will give similar results). SoftMax® Pro software automatically calculated average RFU, standard deviation, and %CV for each set of standard replicates. A standard curve was plotted using the log-log curve fit in the SoftMax Pro software. (See Figure 1.) Sensitivity down to 63 pg/mL was observed using the 96-well microplate format and standard limit of detection calculation of three times standard deviation of the blank. This is well below the lower limit of 250 pg/mL stated in the Quant-iT PicoGreen Assay product insert. Figure 2 shows the lower end of the standard curve. Linearity was excellent throughout the standards' range.

Conclusion

The Quant-iT PicoGreen dsDNA Assay, when run on a SpectraMax Microplate Reader with SoftMax Pro Software, is a quick, sensitive detection method for double-stranded DNA. The analysis capabilities of SoftMax Pro software provide quantitation in an easy-to-read, user customizable report format. A pre-configured protocol is available in the software to facilitate rapid assay setup.

Additional solutions from Molecular Devices

SoftMax Pro GxP Software provides additional tools and features for users who must demonstrate GLP/GMP compliance with FDA 21 CFR Part 11 requirements. Optional hardware and software validation tools are available to speed and simplify the validation process by making it easier to demonstrate compliance of data collection and analysis.

For increased throughput requirements, Molecular Devices' StakMax® microplate handling system integrates with SpectraMax Readers and enables automated processing of batches of 20, 40, or 50 microplates.

For users who need to detect extremely low levels of DNA, Molecular Devices offers the Threshold® system and Threshold Total DNA Assay Kit. The Threshold system can detect and quantitate, at picogram levels, contaminants including total DNA, host cell proteins, bovine contaminants (e.g., BSA, IgG, insulin, transferrin), Proteins A and G, and any unique protein that can be bound by antibodies.

For more information, please visit www.moleculardevices.com/home.html

Reference

1. Molecular Probes Data Sheet MP 07581 (20 December 2005): Quant-iT PicoGreen dsDNA Reagent and Kits.

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