SpectraMax Quant AccuBlue Pico dsDNA Assay Kit

The SpectraMax® Quant™ AccuBlue™ Pico dsDNA Assay Kit is a part of the SpectraMax Quant family of double-stranded DNA (dsDNA) assay products. Each product is tailored to a specific range of DNA concentrations, and is optimized for use with fluorescence microplate readers such as the SpectraMax® microplate readers. Preconfigured protocols are included in SoftMax® Pro Software to make DNA quantitation analysis with the assay kits part of a simplified workflow.

The SpectraMax Quant AccuBlue Pico dsDNA Assay Kit enables quantitation of low concentrations of dsDNA. The linear range of the assay is between 5 pg per well and 3 ng per well in the 96-well microplate format as determined using a fluorescence microplate reader.

Table 1-1: Available Kits

Assay Kit	Explorer Kit	Bulk Kit
SpectraMax® Quant™ AccuBlue™ Pico dsDNA Assay Kit	R8354	R8355

Contents

For research use only. Not for use in diagnostic procedures.

Molecular Devices Patents: http://www.moleculardevices.com/productpatents

Product manufactured for Molecular Devices, LLC
1311 Orleans Drive, Sunnyvale, California, United States of America 94089.
Molecular Devices, LLC is ISO 9001 registered.

Chapter 1: About the SpectraMax Quant AccuBlue Pico dsDNA Assay Kit

The SpectraMax Quant AccuBlue Pico dsDNA Assay Kit provides a convenient tool for quantitation of low amounts of DNA. The key features of this kit are the following:

- Optimal for low concentration dsDNA samples for next-generation sequencing and quantitation of precious or highly fragmented dsDNA from forensic or archaeological samples
- Highly selective for dsDNA over single-stranded DNA or RNA
- Optimized for fluorescence microplate readers, such as SpectraMax® readers
- Simplified data acquisition and analysis with preconfigured protocol in SoftMax® Pro Software

Assay Principles

The SpectraMax Quant AccuBlue Pico dsDNA Assay Kit provides an accurate and sensitive method for quantitation of low amounts of DNA. The kit contains a fluorescent dye that binds to dsDNA. An enhancer is added to reduce background and increase the dynamic range of the assay. Because of the low background, the kit is ideal for use in quantifying DNA from low concentration or precious samples, and for sensitive applications such as next-generation sequencing (NGS) or digital PCR. Figure 1-1 shows the fluorescence excitation peak at 468 nm and the emission peak at 507 nm. Figure 1-2 shows that, unlike absorbance-based measurements, AccuBlue Pico dye is highly selective for dsDNA over single-stranded DNA (ssDNA) or RNA. As shown in Example Standard Curve on page 11, the assay is linear between 5 pg and 3 ng of dsDNA per well in a 96-well microplate format using plate readers such as the SpectraMax® i3x Multi-Mode Microplate Reader.

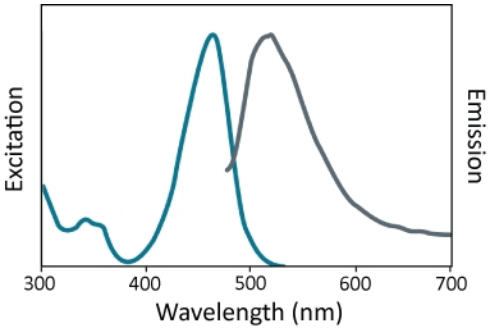

Figure 1-1: SpectraMax Quant AccuBlue Pico dsDNA assay dye fluorescent excitation and emission spectra

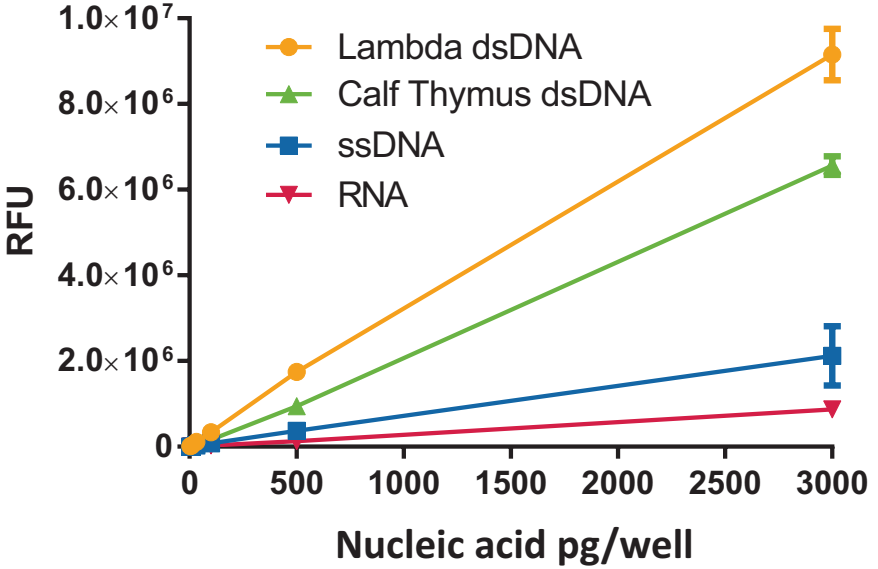

Figure 1-2: SpectraMax Quant AccuBlue Pico dsDNA specificity assay

Specificity of SpectraMax Quant AccuBlue Pico dsDNA assay is demonstrated by comparing the fluorescent signals collected from the same concentrations of lambda dsDNA, calf thymus dsDNA, ssDNA, and RNA. Plots of relative fluorescence units (RFU) vs. nucleic acid concentration show that the signals are significantly higher for calf thymus dsDNA and lambda dsDNA than for RNA and ssDNA.

Chapter 2: Materials and Equipment

Kit Components

Table 2-1: Components of the SpectraMax Quant AccuBlue Pico dsDNA Assay Kit

Item	Explorer Kit (R8354)	Bulk Kit (R8355)
AccuBlue Pico Dye, 400X	1 x 100 µL	1 x 500 µL
AccuBlue Pico Buffer, 20X	2.5 mL	12.5 mL
AccuBlue Pico Enhancer, 100X	1 mL	2 x 1 mL
dsDNA standard from calf thymus, 10 ng/µL	1 x 0.5 mL	2 x 0.5 mL

- The Explorer kit is sufficient for two 96-well microplates.
- The Bulk kit is sufficient for ten 96-well microplates.

The number of microplates is based on the example protocol that is detailed in this document.

Note: The concentration of the dsDNA standard included in this kit (10 ng/µL) is different than the concentration of the dsDNA standard (300 pg/µL) included in the previous version of this kit. The standard was changed due to DNA adsorption issues. Please refer to Assay Setup on page 8 for updated instructions on preparing the standard curve.

Storage and Handling

Store at 4°C. When stored as directed, the kit is stable for at least 6 months from the date it is received.

WARNING! Reagents can contain chemicals that are harmful. Exercise care when handling reagents as described in the related safety data sheet (SDS or MSDS). The safety data sheet is available in the Knowledge Base on the Molecular Devices support web site: www.moleculardevices.com/support

Materials Required but Not Provided

Table 2-2: Reagents and Supplies

Item	Suggested Vendor
Solid black microplates (96-well)	Greiner Bio-One or equivalent
Deionized water for buffer dilution	

Compatible Molecular Devices Microplate Readers

- SpectraMax® i3x Multi-Mode Microplate Reader
- SpectraMax® M2 and M2e Multi-Mode Microplate Readers
- SpectraMax® M3 Multi-Mode Microplate Reader
- SpectraMax® M4 Multi-Mode Microplate Reader
- SpectraMax® M5 and M5e Multi-Mode Microplate Readers
- SpectraMax® Paradigm® Multi-Mode Microplate Reader
- FlexStation® 3 Multi-Mode Microplate Reader
- Gemini™ EM and XPS Microplate Readers
- FilterMax™ F3 and F5 Multi-Mode Microplate Readers

For information about setting up your instrument, see Fluorescence Microplate Reader Setup with SoftMax Pro Software on page 8.

Chapter 3: Assay Protocol

Example of Assay Protocol

Optimal assay conditions for different dsDNA types and plate formats can vary. Molecular Devices recommends verifying the following to determine the optimal conditions for your assay system.

- Because calf thymus DNA is double-stranded, highly polymerized, and approximately 58% AT (42% GC), it can serve as a reference for most plant and animal DNA. Lambda dsDNA yields similar results. You might want to use a standard similar to your unknown samples in DNA length, structure (for example, linear vs. circular), or GC content. Because GC content varies widely depending on the species, a species-specific standard might be appropriate for bacterial DNA.
- Because of variation among plate readers, optimization of instrument settings might be needed for best assay performance. Instrument settings and other factors that can affect linearity and the fluorescence signal include the following:
  - Excitation and emission wavelengths and bandwidths
  - Cut-off filters
  - Sensitivity (gain) settings
  - Pipetting accuracy
  - Microplate manufacturer
- Several methods for isolation of DNA from cells or organisms are available. As a result, different types of contaminants can be present in the samples which can cause either an increase or a decrease in the fluorescent signal. Table 3-1 shows the effects of common DNA contaminants on the fluorescent signal in the SpectraMax Quant AccuBlue Pico dsDNA assay. Triplicate samples of 500 pg dsDNA per well were assayed in the presence of the contaminants at the indicated final concentrations.

Table 3-1: Effects of common DNA contaminants on the fluorescent signal in the SpectraMax Quant AccuBlue Pico dsDNA assay

Compound	Initial concentration in sample	Final concentration in assay (200 µL)	% Change in signal (+/-)
Sodium chloride	500 mM	25 mM	-7%
Magnesium chloride	100 mM	5 mM	-30%
Sodium acetate	600 mM	30 mM	-11%
Ethanol	20%	1.0%	-8%
Phenol	2%	0.10%	-10%
SDS	0.20%	0.01%	-87%
SDS	O.02%	0.001%	-13%
Triton X-100	0.20%	0.01%	-18%
Triton X-100	0.02%	0.001%	-8%
Tween-20	0.10%	0.005%	-8%
dNTPs	2 mM	100 µM	-1%
BSA (500 pg/well std)*	0.8 mg/mL	0.04 mg/mL	-11%
BSA (3000 pg/well std)*	0.8 mg/mL	0.04 mg/mL	-19%

*Not compatible with dsDNA quantitation below 500 pg/well.

Fluorescence Microplate Reader Setup with SoftMax Pro Software

Table 3-2 displays typical fluorescence microplate reader settings. In SoftMax® Pro Software, use the preconfigured SpectraMax Quant AccuBlue Pico dsDNA protocol that is available in the protocol library in the software or on the protocol sharing web site (www.softmaxpro.org).

Table 3-2: SpectraMax Quant AccuBlue Pico dsDNA assay protocol settings

Parameter	Setting
Read Mode	Fluorescence
Read Type	Endpoint
Wavelengths	Excitation: 468 nm with 9 nm bandwidth Emission: 507 nm with 15 nm bandwidth
PMT and Optics	PMT Gain: Automatic Flashes per read: 10 Read From Top

Example of Assay Protocol

The following method was used for generating a standard curve in a 96-well format.

Assay Setup

Use the following procedure to set up the assay in a 96-well plate format:

1. Allow all components to reach room temperature before use.

   Tip: AccuBlue Pico dye is provided in DMSO, which can freeze during storage at 4°C. All kit components can be placed in a 37°C water bath for rapid warming. Allow solutions to cool to room temperature before using.

2. To minimize reagent loss in the cap, before removing the required volume, shake or vortex each component well, and then centrifuge vials briefly.
3. AccuBlue Pico buffer is supplied at 20X. Dilute the buffer to 1X with deionized water on the day of use.
4. To prepare a set of DNA standards, dilute the 10 ng/µL dsDNA standard in the 1X AccuBlue Pico buffer as shown in Table 3-3: Preparation of dsDNA Standards on page 9.

   Note: Prepare the standards fresh the day of the assay. Volumes can be scaled as necessary.

   Tip: DNA in low concentrations such as in this assay range (0.5-300 pg/uL) is susceptible to loss due to adsorption and/or denaturation when stored in polypropylene tubes. For best results, standards should be diluted shortly before using. Using low-binding tubes can also help prevent adsorption.

Table 3-3: Preparation of dsDNA Standards

Standard	Final Concentration	Standard Volume	1X AccuBlue Pico Buffer Volume
A	300 pg/µL	12 µL of AccuBlue Pico Standard (10 ng/µL)	388 µL
B	50 pg/µL	10 µL of A	50 µL
C	10 pg/µL	12 µL of B	48 µL
D	2 pg/µL	12 µL of C	48 µL
E	0.5 pg/µL	15 µL of D	45 µL
F	0 pg/µL	None	60 µL

Note: When following the assay protocol that is described here, the linear range of the assay is determined to be between 5 pg/well and 3 ng/well. Depending on the microplate reader and assay volume, accuracies of 1 pg/well or less might be obtainable. If lower concentration standards are required, then you can prepare 0.25 pg/µL or 0.1 pg/µL standards. For the 0.25 pg/µL standard, combine 5 µL of the 2 pg/µL standard with 35 µL of 1X AccuBlue Pico buffer. For the 0.1 ug/µL standard, combine 10 µL of the 0.5 pg/µL standard with 40 µL of the 1X AccuBlue Pico buffer. Use 10 µL of these standards per well in the assay to obtain 2.5 pg and 1 pg data points.

5. Prepare the working solution as follows on the day of the assay:
   - Dilute the AccuBlue Pico dye 1:400 in 1X AccuBlue Pico buffer in a plastic container (do not use glass), and then vortex or shake to mix well.
   - Dilute the AccuBlue Pico Enhancer 1:100 in the dye/buffer solution that you just made.

Note: Because 200 µL of working solution is required for each standard and sample that is to be tested, volumes can be scaled as required. Prepare only as much working solution as you plan to use within 24 hours.

6. For each dsDNA standard or unknown DNA sample that is to be tested do the following: Add 10 µL of the standard or sample to a well in a black, 96-well microplate, and then add 200 µL of the working solution and mix.

Note: To test samples in triplicate, prepare three separate wells for each dsDNA standard and three separate wells for each unknown DNA sample.

7. Incubate the microplate at room temperature for 5 minutes in the dark.
8. Measure fluorescence in a fluorescence microplate reader with excitation at 468 nm and emission at 507 nm. See Fluorescence Microplate Reader Setup with SoftMax Pro Software on page 8.

Note: In SoftMax® Pro Software, use the preconfigured SpectraMax Quant AccuBlue Pico dsDNA protocol that is available in the protocol library in the software or on the protocol sharing web site (www.softmaxpro.org).

9. For accurate quantitation, subtract the fluorescence of the blanks from that of the standards prior to plotting the standard curve. If you are using a preconfigured protocol in SoftMax Pro Software, assign the 0 pg/µL standard wells as plate blank.

10. Use the standard curve to calculate the concentrations of the unknown DNA samples. See Example Standard Curve on page 11.

Note: Because the fluorescence signal decreases over time after the DNA and dye are combined, an approximately 15% decrease after 3 hours and an approximately 30% decrease after 6 hours, Molecular Devices recommends that you measure fluorescence within 1 hour of setting up the assay.

Note: If the fluorescence of any of the unknown samples exceeds the linear range, then further dilute the sample and use 10 µL of the diluted sample to do the assay. For consistency, use the same volume of sample in all the wells.

Chapter 4: Data Analysis Example

The first step in data analysis is generation of a DNA standard curve that is used to calculate the concentration of unknown DNA samples. A standard curve is generated by plotting the fluorescence values for the DNA standards on the Y-axis and the standard DNA concentrations on the X-axis. A linear curve fit is applied using the dropdown menu in the graph section of SoftMax Pro Software. From the standard curve, concentrations of unknown DNA samples are interpolated. A preconfigured assay protocol in the Protocol Library of SoftMax Pro Software enables automatic graphing of standards, as well as calculation of unknown DNA sample concentrations from the standard curve.

Example Standard Curve

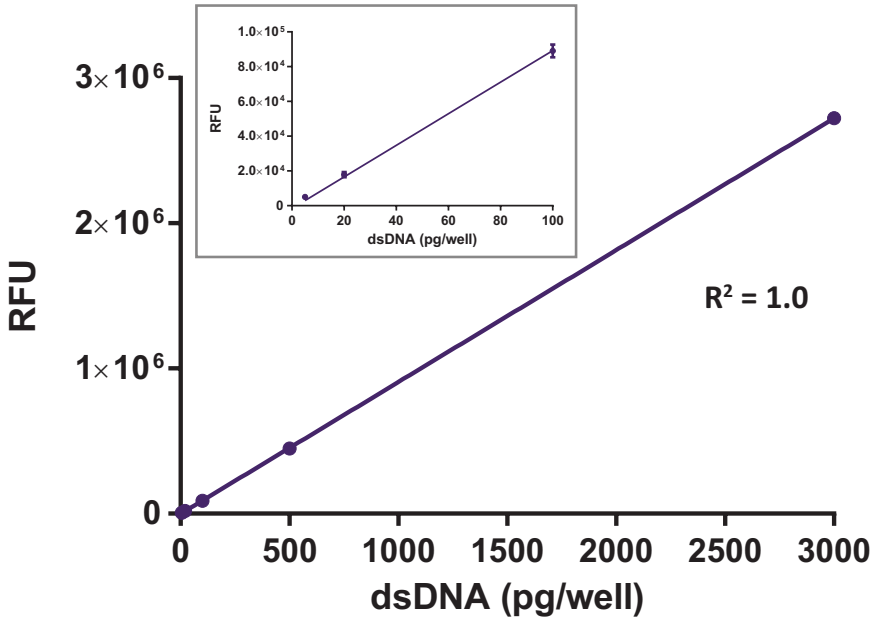

Figure 4-1: SpectraMax Quant AccuBlue Pico dsDNA assay standard curve

Linearity of the SpectraMax Quant AccuBlue Pico dsDNA assay between 5 pg and 3000 pg per well in a 96-well microplate assay with excitation = 468 nm and emission = 507 nm. The inset shows the lower portion of the curve.

Note: The standard curve shown here is for reference only. You must use your own instrument to calculate a standard curve for determining the concentration of DNA in your unknown samples.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest possible level of technical service.

Our support web site, www.moleculardevices.com/support, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you are seeking, follow the links to the Technical Support Service Request Form to send an email message to a pool of technical support representatives.

You can contact your local representative or contact Molecular Devices Technical Support by telephone at 800-635-5577 (North America only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

To find regional support contact information, visit www.moleculardevices.com/contact.

Please have the product name, part number, and lot number available when you call.

SpectraMax Quant Product Family

Table 5-1: SpectraMax Quant Product Family: Available Kits

Assay Kit	Explorer Kit	Bulk Kit
SpectraMax® Quant™ AccuBlue™ Pico dsDNA Assay Kit	R8354	R8355
SpectraMax® Quant™ AccuClear™ Nano dsDNA Assay Kit	R8356	R8357
SpectraMax® Quant™ AccuBlue™ HiRange dsDNA Assay Kit	R8358	R8359

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