Rev. September 4, 2009

MagicBlue™ High Sensitivity dsDNA Quantitation Kit (0.2 – 100 ng)

Catalog Number: 31006

Table 1. Kit Components and Storage

Material	Amount	Storage Condition	Stability
A: MagicBlue™ High Sensitivity dsDNA Quantitation Solution	2 x 125 mL (250 mL total)	Kit components should be stored at 4°C. *Protect from light.*	Kit components are stable for at least 6 months if stored as directed.
B: MagicBlue™ (100X) High Sensitivity Enhancer	3 X 1 mL		
C: dsDNA Standards (calf thymus)	Set of 8 (500 uL each): 0, 0.5, 1, 2, 4, 6, 8 and 10 ng/uL		
Number of Assays: 1,000 with a 200 uL assay volume.			
Recommended excitation/emission maxima: Best linearity is achieved at Ex/Em of 485nm/530nm.			

Product Description

The High Sensitivity dsDNA Quantitation Kit provides ease and simplicity for DNA quantitation. The kit contains MagicBlue™ dsDNA Quantitation Solution, Enhancer and pre-diluted dsDNA standards. This quantitation kit is highly reliable in detecting dsDNA ranging from 0.2 to 100 ng (See Figure 2), and offers advantages in stability, linear dynamic range, and sensitivity over other traditional methods of DNA quantitation. The assay kit is tolerable to common contaminants such as proteins, salts, organic solvents and detergents. See the appendix table for more information. The assay can be adapted for use in microplates, tubes or cuvettes.

General Protocol for Using the DNA Quantitation Assay Kit

The MagicBlue™ High Sensitivity dsDNA Quantitation Kit is used with fluorescence 96-well plate readers equipped with fluorescein excitation and emission filters or a fluorometer such as the Qubit™ (Invitrogen). Although the MagicBlue High Sensitivity reagent does not readily enter cells, we advise that the reagent be treated with the safety precautions as other potentially harmful reagents and to dispose of the reagent in accordance with local regulations. The assay is performed at room temperature. Centrifuge the dsDNA standards before opening vials to minimize loss on the cap. Use properly calibrated pipets for best accuracy.

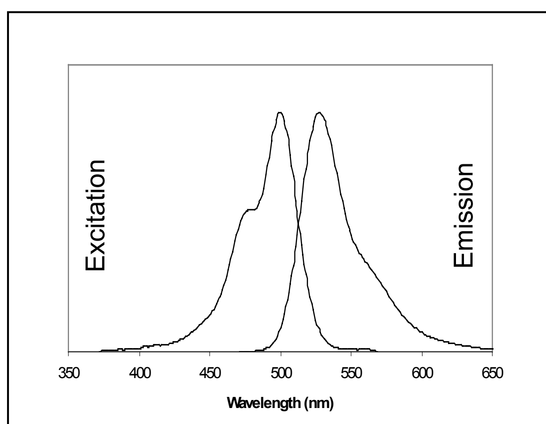

Figure 1: Excitation and emission spectra for MagicBlue™ High Sensitivity dsDNA quantitation reagent in the presence of dsDNA.

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1. MagicBlue™ High Sensitivity dsDNA Quantitation Assay Using a Fluorescence Microplate Reader

1.1. Remove the DNA quantitation kit from storage and allow the kit's components to warm to room temperature. Invert the quantitation solution bottle several times and vortex the enhancer vial before removing needed aliquots. If precipitation is seen in the enhancer, warm up the vials in a water bath and vortex until dissolved.

1.2. For each 96 well plate, add 200 uL of 100X MagicBlue Enhancer to 20 mL of MagicBlue Quantitation Solution to prepare the working solution. Prepare the working solution right before use and mix only what you plan to use as precipitation may occur over time.

1.3. Add 200 uL of the working MagicBlue solution into each of the separate wells into a black 96-well microplate as needed. Accurate multi-channel pipets and reservoirs can be used to facilitate this process. Black plates are recommended to minimize fluorescence bleed-through from other wells. We recommend all black 96-well plates from Greiner Bio-one or Corning as they have shown give the most consistent signal-to-noise sensitivity at low DNA concentrations.

1.4. Add 10 uL of each of the dsDNA standards into separate wells and mix well by pipeting up and down. Be careful not to introduce any nucleases into the vials of the DNA standards when pipeting aliquots for the assay. It is recommended that there be duplicates or triplicates of the DNA standards. It is also recommended to have a standard curve on each 96-well plate that is used to minimize variability between plates.

1.5. Add 10uL of the unknown DNA into each of the separate wells and mix well by pipeting up and down. It is recommended that there be duplicates or triplicates of the unknown DNA samples.

1.6. Incubate the microplate at room temperature for 15-30 minutes in the dark if possible.

1.7. Measure the fluorescence using a microplate reader with 485 nm excitation and 530 nm emission parameters.

1.8. Generate a standard curve to determine the unknown DNA concentration. For the DNA standards, plot the amount of DNA vs. Fluorescence, and fit a straight line through these points. If desired, the 0 standard fluorescence can be subtracted from the values for a zero y-intercept.

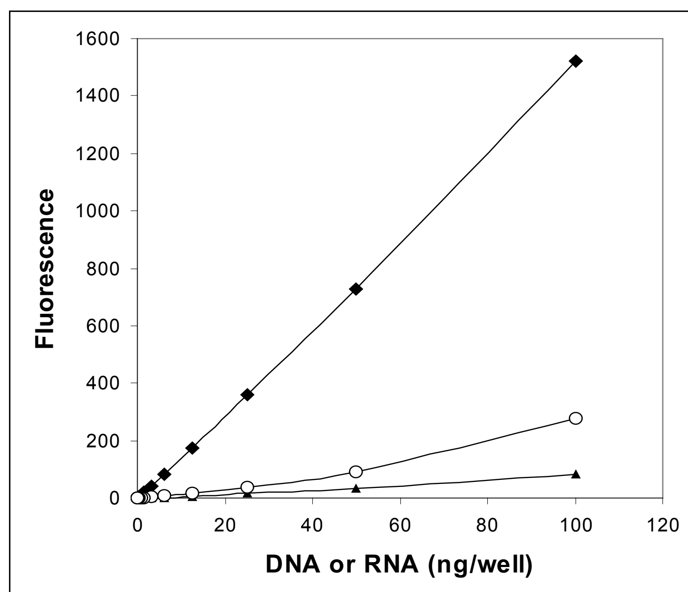

Figure 2: MagicBlue High Sensitivity dsDNA Quantitation kit selectivity and sensitivity for dsDNA. Triplicate samples of calf thymus dsDNA (♦), total mouse liver RNA (O) or viral M13mp18 single-stranded DNA (▲) were assayed using MagicBlue and read at 485/530nm. The total fluorescence was plotted against the amount of DNA or RNA. The background was subtracted from the fluorescence values.

2. **MagicBlue™ High Sensitivity dsDNA Quantitation Assay Using the Qubit™ Fluorometer from Invitrogen**

2.1. Remove the DNA quantitation kit from storage and allow the kit's components to warm to room temperature.

2.2. Add 100 uL of 100X MagicBlue Enhancer to 10 mL of MagicBlue Quantitation Buffer or fractions thereof. It is best to mix the reagents right before use and make only what you plan to use as precipitation may occur over time.

2.3. Add 200 uL of the solution into each assay tube.

2.4. Add 10 uL of the 0 and 10 ng/uL dsDNA standards into two of the assay tubes (for calibration).

2.5. Add 10 uL of the unknown samples into assay tubes and vortex or flick well.

2.6. Incubate the samples at room temperature for 15-30 minutes.

2.7. Measure the fluorescence on the Qubit™ fluorometer using the High Sensitivity program. Consult the instruction manual for guidance on using the Qubit™ or a substitute fluorometer.

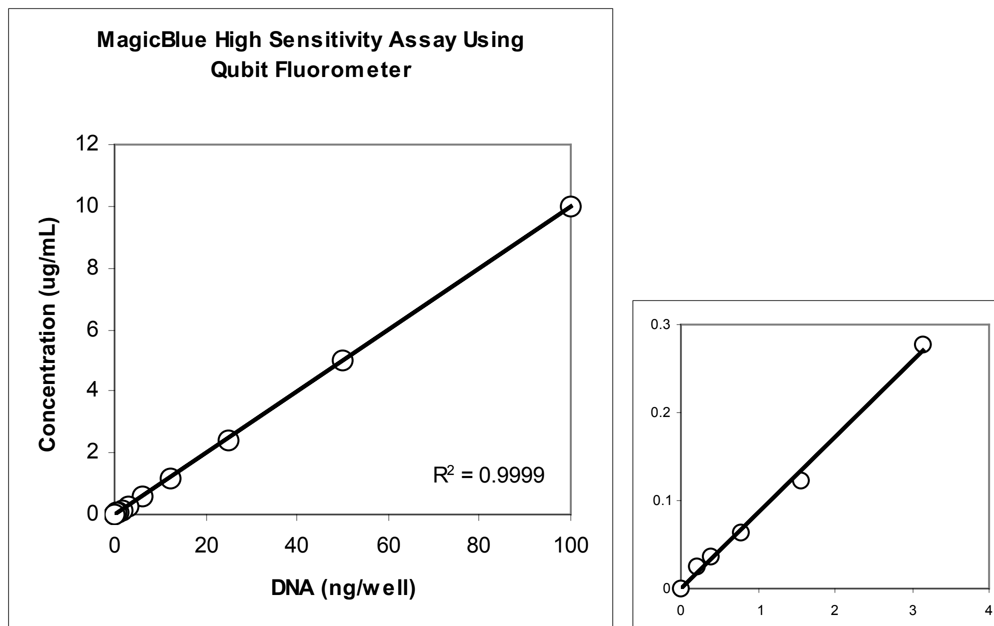

Figure 3: MagicBlue™ dsDNA quantitation using the High Sensitivity Qubit™ program with 0 and 100 ng calibration standards and 10 uL samples of two-fold dilutions of dsDNA.

Considerations for Data Analysis

Calf thymus DNA can often serve as a reference for most plant and animal DNA because it is double-stranded, highly polymerized and is approximately 58% AT (42% GC). At times it is preferable to use a dsDNA standard similar to the unknown samples (i.e. similar in size, linear vs. circular). We have found that most linear dsDNA, yield similar results; however, it is best to compare the concentration of the unknown sample to a more appropriate standard if necessary. If the fluorescence of an unknown sample is higher than the linear range, further dilute the sample and add 10 uL of the diluted sample to perform the assay. For consistency, it is best to use the same volume in all the wells with samples that do not have high levels of contaminating substances.

Fluorescence quantitation by the MagicBlue™ High Sensitivity reagent is linear from 0.2 – 100 ng dsDNA. The dynamic range can be extended to 200 ng with some distortion of the standard curve between 100 and 200 ng. For best results, subtract the background values so that the standard curve goes through zero on the y-intercept. For example, if triplicates were done for the DNA samples, take the average of the three fluorescence values. Then, subtract the average fluorescence value of the working solution without any DNA (0 standard) from the average DNA standard values. Plot this value (the DNA fluorescence value minus the background) against the standard DNA amount in each well. The fluorescence values correspond to the amount of DNA in the working solution. It is necessary to take into account the dilution factor to finally obtain the concentration of the sample. If lower end standards are desired, you can further dilute any of the standards with 1X TE to 0.02 ng/uL. Use 10 uL/well to obtain a 0.2 ng/well standard.

Due to differences in instruments, check instrument settings to optimize for the best linearity. Some factors that can affect the final linearity and relative fluorescence intensity are: (1) the excitation and emission wavelengths and bandwidths, (2) cut-off filters, (3) sensitivity settings, (4) pipet accuracy, and (5) microplate manufacturers.

Appendix

Table 2. Effects of Contaminants in the MagicBlue High Sensitivity dsDNA Assay

Contaminant	Final Concentration in Assay	Concentration in 10 uL Sample	Result
Salts			
Ammonium Acetate	5 mM	100 mM	Pass
Sodium Acetate	30 mM	600 mM	Pass
Sodium Chloride	10 mM	200 mM	Pass
Magnesium Chloride	1.25 mM	25 mM	Pass
Organic Solvents			
Phenol	0.1 %	2 %	Pass
Ethanol	0.5 %	10 %	Pass
Chloroform	0.1 %	2 %	Pass
Detergents			
Sodium Dodecyl Sulfate	0.01 %	0.2 %	Pass
Triton X-100	0.01 %	0.2 %	Pass
Proteins			
Bovine Serum Albumin	10 mg/mL	200 mg/mL	Pass †
Other Compounds			
dNTPS *	100 uM	2 mM	Pass
Polyethylene Glycol	2 %	40%	Pass
Agarose	0.1 %	2%	Pass

Triplicate samples of 100 ng of ds DNA were assayed in the presence or absence of the contaminants at the indicated final concentrations. In the majority of the cases, a pass indicates that there was < 20% change from the assay in the absence of the contaminant. Samples were excited at 485 nm and fluorescence intensity was measured at 530 nm on a Molecular Devices Gemini XS microplate reader.

† indicates a pass but with some perturbation of the standard curve.
* dNTPs were a mixture of dATP,dGTP,dCTP, and dTTP.

Please download the MagicBlue Flyer from the Biotium website (www.biotium.com) for more detailed information.
MagicBlue is a trademark of Biotium, Inc.; PicoGreen, Qubit and Quant-iT are trademarks of Invitrogen Corp.

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