

MagicBlue™ Broad Range dsDNA Quantitation Kit (2 - 2000ng)

Catalog Number: 31007

Table 1. Kit Components and Storage

Material	Amount	Storage Condition	Stability
A: MagicBlue™ Broad Range dsDNA Quantitation Solution	2 x 125 mL (250 mL total)	Kit components should be stored at 4°C. <i>Protect from light.</i>	Kit components are stable for at least 6 months if stored as directed.
B: MagicBlue (100X) Broad Range Enhancer	3 X 1 mL		
C: dsDNA Standards (calf thymus)	Set of 8 (500 uL each): 0, 2, 5, 10, 20, 50, 100 and 200 ng/uL		
Number of Assays: 1,000 with a 200 uL assay volume.			
Recommended fluorescence excitation/emission maxima: 350/460nm (in the presence of dsDNA).			

Product Description

The MagicBlue™ Broad Range Quantitation Kit provides ease and simplicity for DNA quantitation. The kit contains the MagicBlue™ Broad Range dsDNA Quantitation Solution, Enhancer and pre-diluted dsDNA standards. This quantitation kit is highly sensitive in detecting dsDNA ranging from 2 to 2000 ng (See Figure 2), and offers advantages in a large dynamic range and high 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.

General Protocol for Using the DNA Quantitation Assay Kit

The MagicBlue™ Broad Range dsDNA Quantitation Kit is used with fluorescence 96-well plate readers equipped with proper UV excitation and emission filters. 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. Warm up all reagents to room temperature and perform the assay at room temperature. Centrifuge the dsDNA standards before opening vials to minimize loss on the cap. Use properly calibrated pipets for best accuracy.

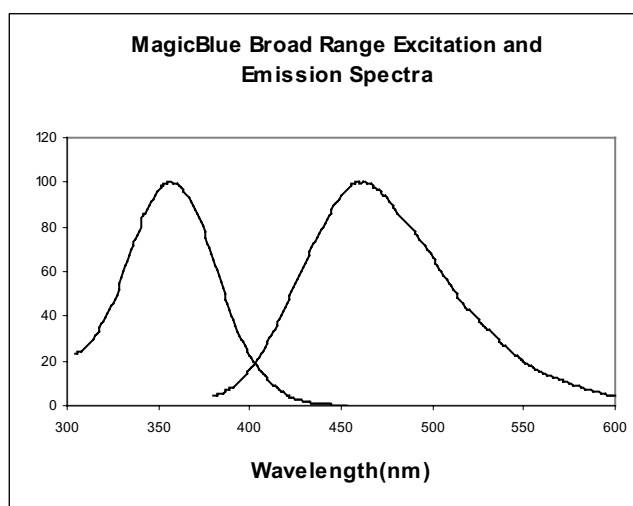


Figure 1: Excitation and emission spectra for MagicBlue™ Broad Range dsDNA quantitation reagent in the presence of excess dsDNA.

1. MagicBlue™ Broad Range 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 μ L 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 μ L of the MagicBlue working 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 μ L of each of the dsDNA standards into each of the 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 out aliquots for the assay. It is recommended that there be 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 10 μ L of the unknown DNA into each of the separate wells and mix well by pipeting up and down. It is recommended that there be triplicates of the unknown DNA samples.
- 1.6. Incubate the microplate at room temperature for 15-30 minutes in the dark.
- 1.7. Measure the fluorescence using a microplate reader with 350 nm excitation and 460 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 trend line through these points. If desired, the 0 standard fluorescence can be subtracted from the values for a zero y-intercept.

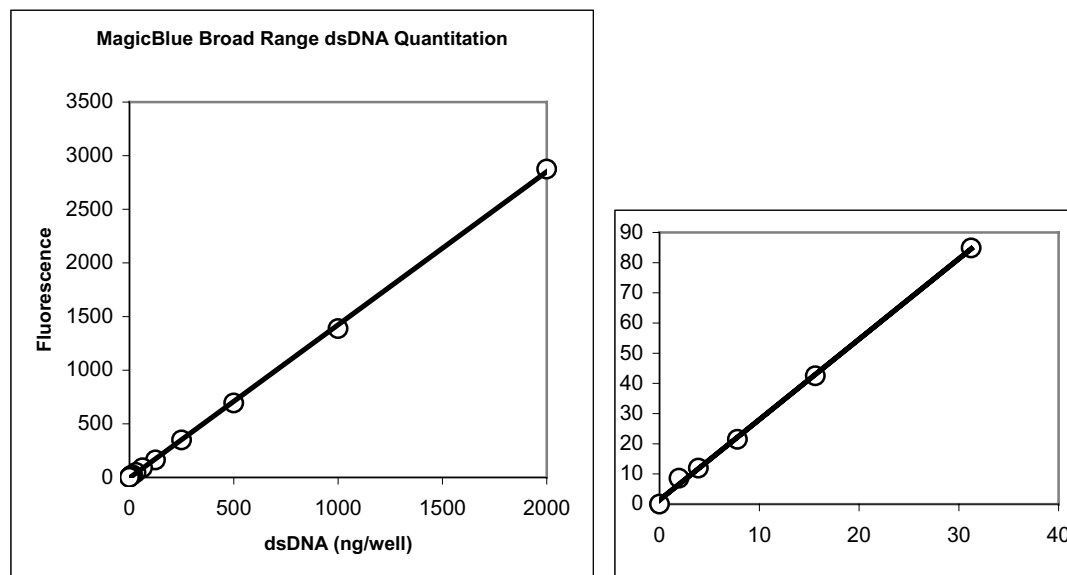


Figure 2: MagicBlue™ Broad Range dsDNA Quantitation kit linearity from 2 – 2000ng/well. The smaller graph to the right is the lower range of the DNA titration. Duplicates of dsDNA in two-fold dilutions were assayed in 96-well plates and read at 350/460nm on a Molecular Devices Gemini XS plate reader. The average background value (0 DNA standard) was subtracted from the average fluorescence values and plotted with a trend line. Fluorescence quantitation by the MagicBlue™ Broad Range reagent is linear from 2 - 2000 ng dsDNA but can be extended to 4000 ng with some distortion of the standard curve.

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). For bacterial DNA, a different standard may be needed because the AT% varies widely depending on the species. At times it is preferable to use a dsDNA standard similar to the unknown samples (similar in size, linear vs. circular). We have found that most linear dsDNA yield similar results (see Figure 3 below); however, it is best to use a more appropriate standard if necessary. If the fluorescence of any of the unknown samples is higher than the linear range, further dilute the sample and add 10 μ L of the diluted sample to perform the assay. For consistency, it is best to use the same volume of sample in all the wells. Fluorescence quantitation by the MagicBlue™ Broad Range reagent is linear from 2 - 2000 ng dsDNA but can be extended to 4000 ng with some distortion of the standard curve (See Fig. 4). 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 0.2 ng/ μ L. Use 10 μ L/well to obtain a 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.

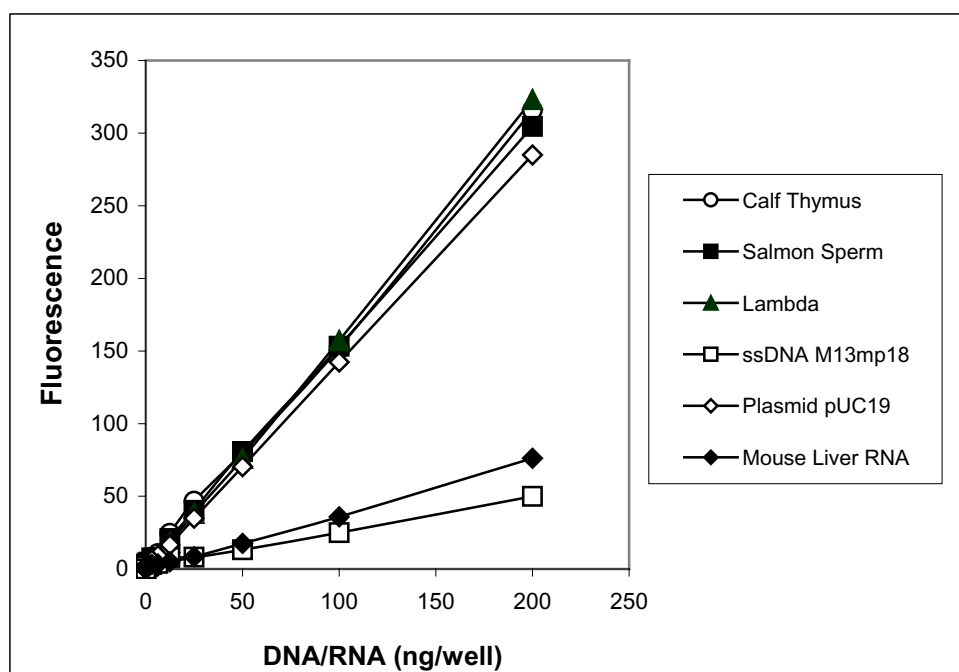


Figure 3. Relative fluorescence intensities of different nucleic acids using the MagicBlue Broad Range dsDNA Quantitation kit.

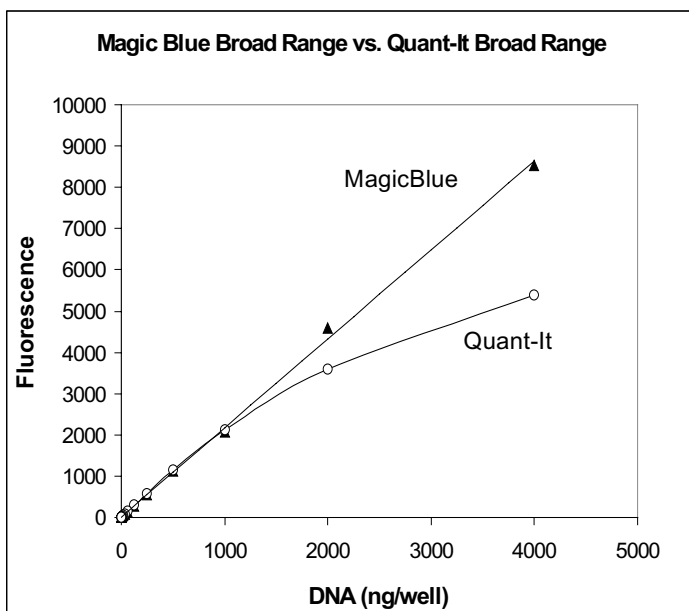


Figure 4: Two-fold dilutions of calf thymus DNA were assayed using MagicBlue™ or Quant-iT™ Broad Range assay kits. MagicBlue has improved linearity and wider dynamic range than the Quant-iT Broad Range kit.

APPENDIX

Table 2. Effects of Contaminants in the MagicBlue Broad Range dsDNA Assay

Contaminant	Final Concentration in Assay	Concentration in 10 uL Sample	Result
Salts			
Ammonium Acetate	5 mM	100 mM	Pass
Sodium Chloride	50 mM	1 M	Pass
Organic Solvents			
Ethanol	0.5%	10 %	Pass
Phenol	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	1 mg/mL	20 mg/mL	Pass [†]
Other			
dNTPS *	100 uM	2 mM	Pass

Triplicate samples of 2000 ng of dsDNA were assayed in the presence or absence of the contaminants at the indicated final concentrations. In the majority of cases, a pass indicates that there was < 20% change from the assay in the absence of the contaminant. Samples were excited at 350 nm and fluorescence intensity was measured at 460 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.; Quant-iT is a trademark of Invitrogen Corp.

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