AccuBlue^{TM} Broad Range dsDNA Quantitation Kit (2 – 2000 ng)

Catalog Number: 31007 (1000 assays)

Revised June 3, 2011

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Product Description

The AccuBlue™ (formerly MagicBlue) Broad Range Quantitation Kit provides ease and simplicity for DNA quantitation. The kit contains the AccuBlue™ 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.

Table 1. Kit Components and Storage

Material	Amount	Storage Condition	Stability
A: AccuBlue™ Broad Range dsDNA Quantitation Solution	1 x 250 mL	Store kit components at 4°C, protected from light.	Kit components are stable for at least 6 months if stored as directed.
B: AccuBlue (100X) Broad Range Enhancer	3 X 1 mL		
C: dsDNA Standards (calf thymus)	Set of 9 (500 uL each): 0, 2, 6.25, 12.5, 25, 50, 100, 150,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).			

General Protocol for Using the DNA Quantitation Assay Kit

The AccuBlue™ 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. Heat the quantitation solution and enhancer to 37ºC for at least one hour and shake/vortex well to ensure reagents are in solution. Warm up DNA standards 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 pipettes for best accuracy.

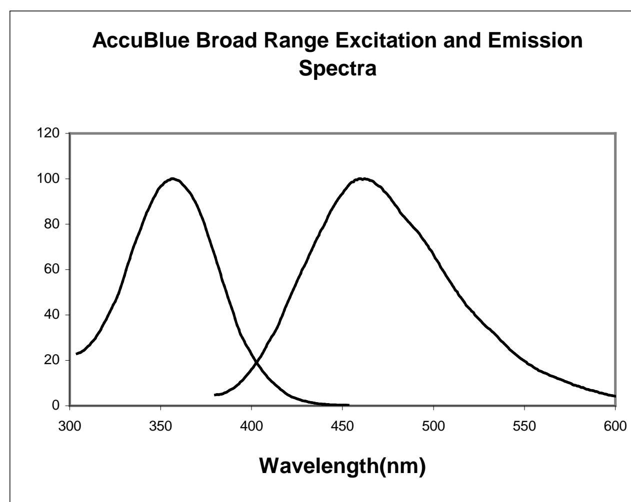

Figure 1: Excitation and emission spectra for AccuBlue™ Broad Range dsDNA quantitation reagent in the presence of excess dsDNA.

1. AccuBlue™ Broad Range dsDNA Quantitation Assay Using a Fluorescence Microplate Reader

1.1. Remove the DNA quantitation kit from storage and heat the quantitation solution and enhancer to 37°C for at least one hour to ensure that the reagents are in solution. Shake the quantitation solution bottle well and vortex the enhancer vial before removing the required volume of solution.

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

1.3. Add 200 uL of the AccuBlue working solution into each of the separate wells into a black 96-well microplate as needed. Accurate multi-channel pipettes and reservoirs can be used to facilitate this process. Black plates are recommended to minimize fluorescence bleed-through between 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 each of the separate wells and mix well by pipetting up and down. Be careful not to introduce any nucleases into the vials of the DNA standards when pipetting out aliquots for the assay. It is recommended to test the DNA standards in triplicate. It is also recommended to include a standard curve on each 96-well plate that is used to minimize variability between plates.

1.5. Add 10 uL of the unknown DNA into each of the separate wells and mix well by pipetting up and down. It is recommended assay unknown DNA samples in triplicate.

1.6. Incubate the microplate at room temperature for 5 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 zero standard fluorescence can be subtracted from the values for a zero y-intercept.

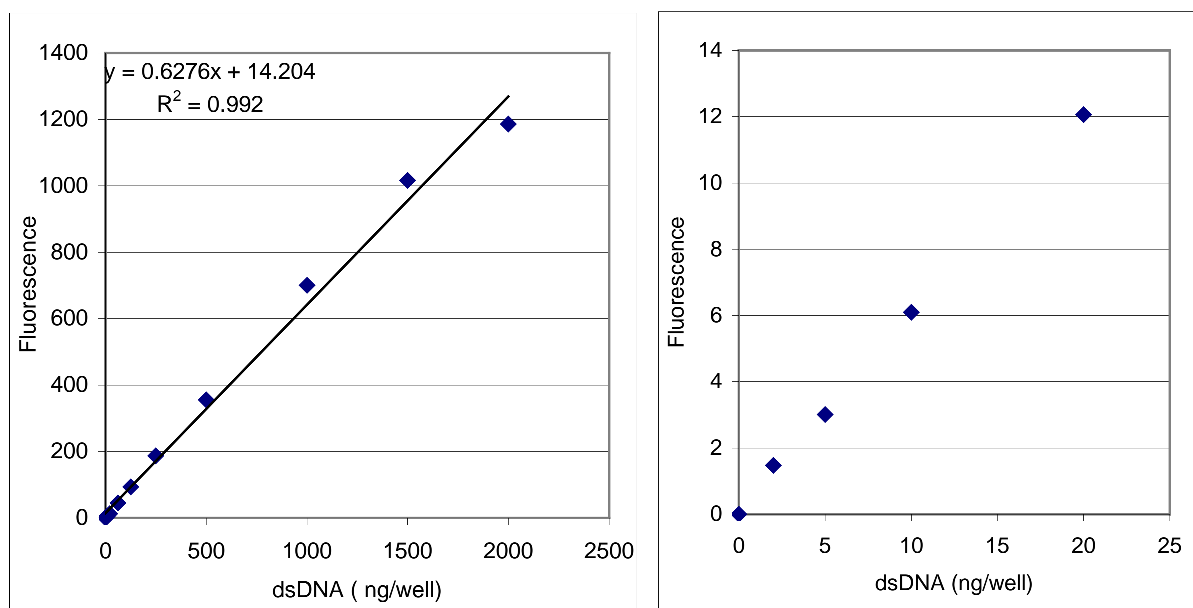

Figure 2:
AccuBlue™ Broad Range dsDNA Quantitation kit linearity from 2 – 2000 ng/well. The smaller graph to the right is the lower range of the DNA titration. Duplicates of dsDNA in two-fold dilutions were assayed in 96-well plates and read at 350/460nm on a Molecular Devices Gemini XS plate reader.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

AccuBlue™ 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 serve as a reference for most plant and animal DNA because it is double-stranded, highly polymerized and is approximately 58% AT (42% GC). We have found that most linear dsDNA yield similar results (see Figure 3 below); however, it may be preferable to use a standard similar to the unknown samples in DNA length, structure (i.e., linear vs. circular), or GC content. For bacterial DNA, a specific species standard may be desired because the GC content varies widely depending on the species. If the fluorescence of any of the unknown samples 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 of sample in all the wells. Fluorescence quantitation by the AccuBlue™ Broad Range reagent is linear from 2 - 1500 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 intersects the y-axis at zero. For example, if triplicates were assayed, take the average of the three fluorescence values. Then, subtract the average fluorescence value of the working solution without 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/uL, and add 10 uL/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) pipetting accuracy, and (5) microplate manufacturers.

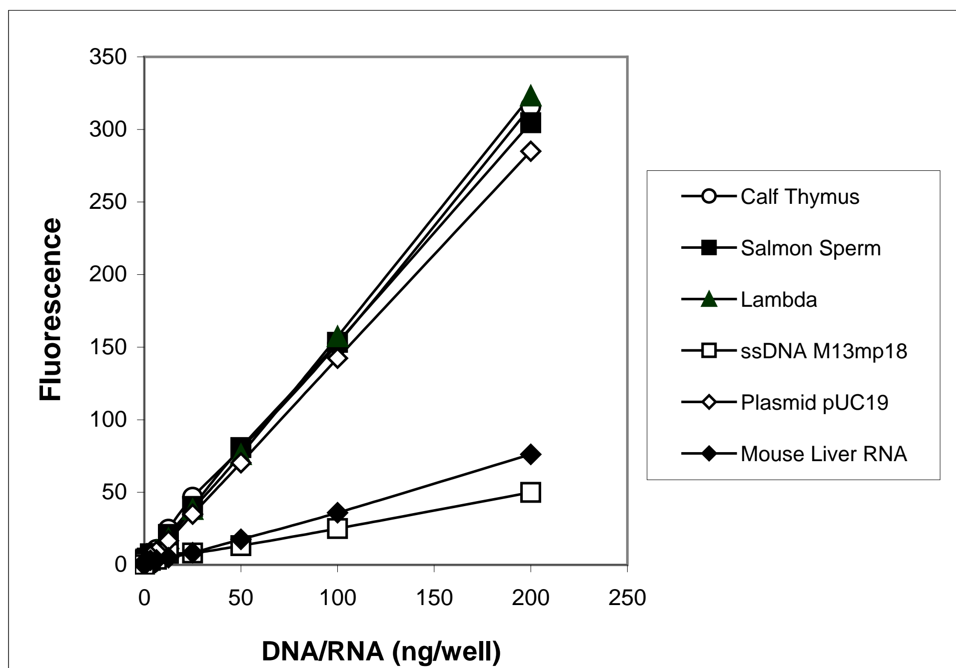

Figure 3. Relative fluorescence intensities of different nucleic acids using the AccuBlue Broad Range dsDNA Quantitation kit.

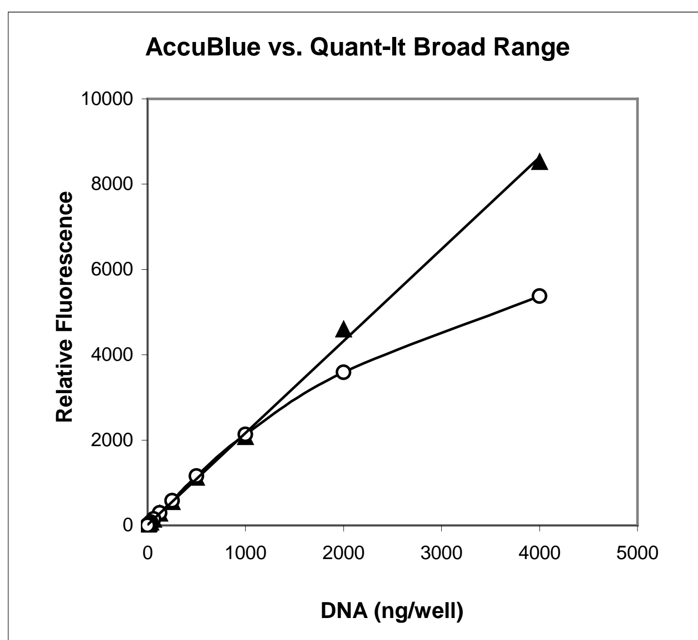

Figure 4: Two-fold dilutions of calf thymus DNA were assayed using AccuBlue™ or Quant-iT™ Broad Range assay kits. AccuBlue has improved linearity and wider dynamic range than the Quant-iT Broad Range kit.

Appendix

Table 2. Effects of Contaminants in the AccuBlue 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.
* A mixture of dATP, dGTP, dCTP, and dTTP.

Please download the AccuBlue Flyer from the Biotium website (www.biotium.com) for more detailed information.
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