



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

Catalog Number: 31006 (1000 assays)

Contact Information

Address: Biotium, Inc.
3159 Corporate Place
Hayward, CA 94545
USA
Telephone: (510) 265-1027
Fax: (510) 265-1352
Email: btinfo@biotium.com
Website: www.biotium.com

Product Description

The AccuBlue™ (formerly MagicBlue) High Sensitivity dsDNA Quantitation Kit provides ease and simplicity for DNA quantitation. The kit contains AccuBlue™ 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.

Table 1. Kit Components and Storage

Material	Amount	Storage Condition	Stability
A: AccuBlue™ High Sensitivity dsDNA Quantitation Solution	1 x 250 mL	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: AccuBlue™ (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.			

General Protocol for Using the DNA Quantitation Assay Kit

The AccuBlue™ High Sensitivity dsDNA Quantitation Kit is used with fluorescence 96-well plate readers equipped with fluorescein excitation and emission filters or fluorimeters such as the Qubit™ (Invitrogen) and QuantiFluor™ (Promega). Although the AccuBlue 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.

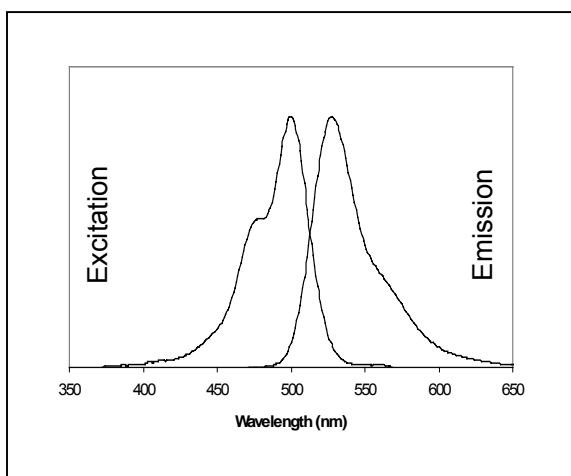


Figure 1: Excitation and emission spectra for AccuBlue™ High Sensitivity dsDNA quantitation reagent in the presence of dsDNA.

1. AccuBlue™ 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 100X AccuBlue Enhancer. If precipitation is seen in the enhancer, warm up the vial in a water bath and vortex until dissolved.
- 1.2. Prepare working solution **IMMEDIATELY** before use. For each 96-well plate, add 200 μ L of 100X AccuBlue Enhancer to 20 mL of AccuBlue Quantitation Solution to prepare the working solution. Mix well and use immediately. Precipitation may occur over time if solution is prepared and allowed to sit before use.
- 1.3. Add 200 μ L of the AccuBlue working solution to each well of a black 96-well microplate. 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 Greiner Bio-one or Corning black 96-well plates as they have shown to 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 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. Duplicates of the DNA standards can be performed for better accuracy, if needed. It is also recommended to have a standard curve on each 96-well plate to minimize variability between plates.
- 1.5. Add 10 μ L of the unknown DNA into each well and mix well by pipeting up and down. It is recommended to run duplicates or triplicates of the unknown DNA samples.
- 1.6. Incubate the microplate at room temperature for 1-5 minutes in the dark.
- 1.7. Measure the fluorescence using a microplate reader with 485 nm excitation and 530 nm emission, with the appropriate cut-off.
- 1.8. Subtract the fluorescence value of the blank from that of each of the samples. Generate a standard curve by plotting fluorescence versus DNA concentration of the DNA standards. Use the standard curve and the fluorescence of the unknown DNA to determine the unknown DNA concentration.

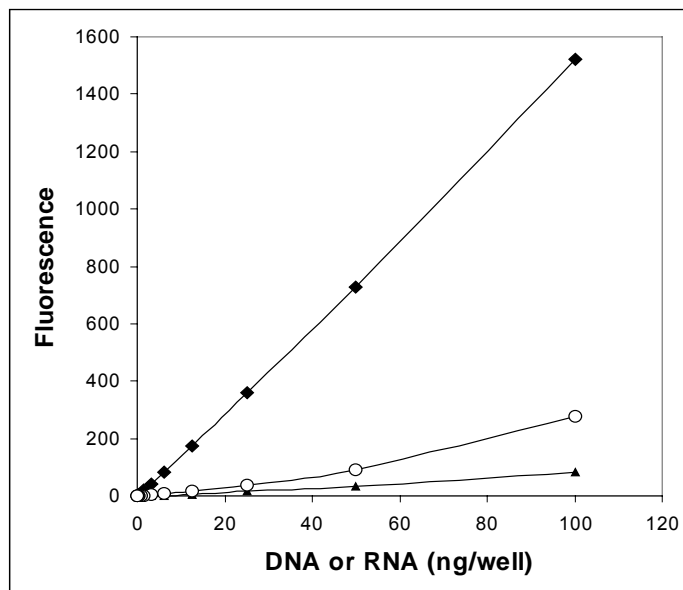


Figure 2: AccuBlue High Sensitivity dsDNA Quantitation kit selectivity and sensitivity for dsDNA. Triplicate samples of calf thymus dsDNA (\blacklozenge), total mouse liver RNA (O) or viral M13mp18 single-stranded DNA (\blacktriangle) were assayed using AccuBlue and read at 485/530nm. The total fluorescence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

2. AccuBlue™ 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. Vortex the 100X Enhancer and shake the Quantitation Solution to mix.
- 2.2. Add 100 μ L of 100X AccuBlue Enhancer to 10 mL of AccuBlue Quantitation Solution or fractions thereof. Mix the reagents **immediately** before use and make only what you plan to use as precipitation may occur over time.
- 2.3. Add 200 μ L of the solution into each assay tube.
- 2.4. Add 10 μ L of the 0 and 10 ng/ μ L dsDNA standards into two of the assay tubes (for calibration).
- 2.5. Add 10 μ L of the unknown samples into assay tubes and vortex or flick well.
- 2.6. Incubate the samples at room temperature for 1-5 minutes.
- 2.7. Measure the fluorescence on the Qubit™ fluorometer using the High Sensitivity program, according to the manufacturer's recommendation.

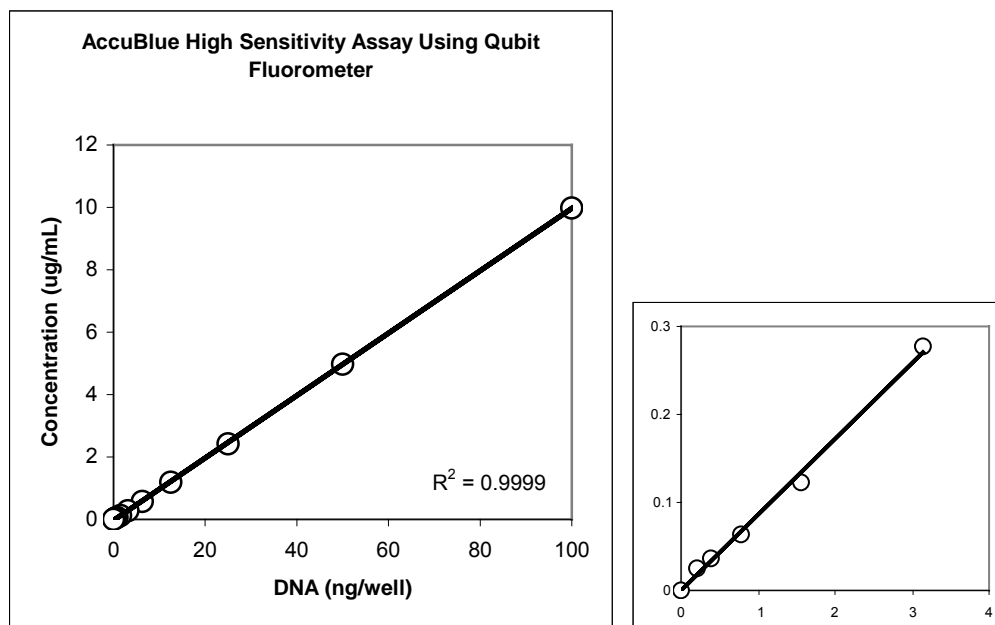


Figure 3: AccuBlue™ dsDNA quantitation using the High Sensitivity Qubit™ program with 0 and 100 ng calibration standards and 10 μ L samples of two-fold dilutions of dsDNA.

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 μ L 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 AccuBlue™ High Sensitivity reagent is linear from 0.2 – 100 ng dsDNA. The dynamic range can be extended to 200 ng; however, there are some distortions of the standard curve between 100 and 200 ng. Be sure to take into account the dilution factor when calculating 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 AccuBlue 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 AccuBlue Flyer from the Biotium website (www.biotium.com) for more detailed information. AccuBlue is a trademark of Biotium, Inc.; PicoGreen, Qubit and Quant-iT are trademarks of Invitrogen Corp; QuantiFluor is a trademark of Promega Corporation.