Biotium
Glowing Products for Science™

3159 Corporate Place
Hayward, CA 94545
www.biotium.com

Revised: February 20, 2014

Product Information

AccuBlue™ High Sensitivity dsDNA Solution

Component	31008-T 200 assays	31008 1000 assays
AccuBlue High Sensitivity dsDNA Quantitation Solution	50 mL 31008-TA	250 mL 99940
AccuBlue High Sensitivity Enhancer (100X)	1 X 1 mL 99942	3 X 1 mL 99942

Storage and Handling

Store kit at 4ºC. Protect quantitation solution from light. The kit is stable for at least 6 months from date of receipt when stored as recommended.

Spectral Properties

Best linearity is achieved at Ex/Em 485/530 nm. See Figure 1 for spectra.

Product Description

The AccuBlue™ High Sensitivity dsDNA Quantitation Solution provides ease and simplicity for DNA quantitation. AccuBlue assays are based on binding of fluorescent DNA dyes that selectively detect double-stranded DNA over RNA or single-stranded DNA (Figure 2), unlike absorbance-based measurements. The assay is highly reliable in detecting dsDNA ranging from 0.2 to 100 ng (Figure 3), and offers advantages in stability, linear dynamic range, and sensitivity over other traditional methods of DNA quantitation. The assay is tolerant of common contaminants such as proteins, salts, organic solvents and detergents (Table 1). In addition, the AccuBlue High Sensitivity DNA dye does not readily enter cells, and is non-toxic and non-mutagenic.

The AccuBlue™ High Sensitivity dsDNA Quantitation Kit is used with fluorescence 96-well plate readers equipped with fluorescein excitation and emission filters. Biotium's AccuLite™ 470 handheld fluorometer is pre-programmed for use with the AccuBlue High Sensitivity assay; the assay also can be used with fluorometers such as the Qubit® (Invitrogen) and QuantiFluor™-P (Promega).

AccuBlue High Sensitivity dsDNA Quantitation Solution also is available in a kit with pre-diluted standards (catalog no. 31006). Biotium also offers AccuBlue Broad Range dsDNA quantitation assay for measuring DNA in the range of 2-2000 ng, and AccuClear™ Ultra High Sensitivity dsDNA quantitation assay for measuring DNA in the range of 0.03-250 ng. See related products for details.

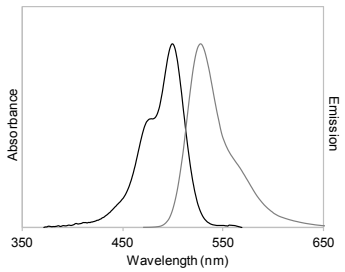

Figure 1: Excitation and emission spectra for AccuBlue™ High Sensitivity dsDNA dye in the presence of dsDNA.

Assay Protocol

Note: see the Appendix for information on using the AccuBlue High Sensitivity assay with the AccuLite 470 fluorometer.

1. Use properly calibrated pipettes and DNase-free pipette tips, tubes and plates for best accuracy. It is recommended to test each DNA standard and each unknown sample in triplicate. If more than one 96 well plate is to be tested in a single assay, it is recommended to include a standard curve on each plate to minimize variability between plates.

2. Prepare DNA standards in 1X TE with the dsDNA of your choice. Prepare a 10 ng/uL stock solution of DNA. Determine the DNA concentration on the basis of absorbance at 260 nm in a cuvette with a 1 cm pathlength. An A_{260} of 0.2 corresponds to a 10 ng/uL dsDNA solution. Perform 2-fold serial dilutions to obtain several standards. Use 1X TE as 0 DNA standard.

3. Allow the kit components to warm to room temperature before use. 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. Before removing the required volume, mix each component well by shaking or vortexing, and centrifuge vials briefly before opening to minimize reagent loss on the cap.

4. Prepare working solution IMMEDIATELY before use. For each 96-well plate, add 200 uL of 100X Enhancer to 20 mL of 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. Volumes can be scaled as needed.

5. For each sample to be tested, pipette 200 uL of the working solution per well of a black 96-well microplate. To test samples in triplicate, prepare three separate wells for each DNA standard and three separate wells for each unknown DNA sample. Accurate multi-channel pipettes and reagent reservoirs can be used to increase throughput. Black plates are recommended to minimize fluorescence bleed-through between wells. We have found that black 96-well plates from Greiner Bio One or Corning give the most consistent signal-to-noise ratio at low DNA concentrations.

6. Add 10 uL of each dsDNA standard into its own separate well containing working solution and mix well by pipetting up and down.

7. Pipette 10 uL of each unknown DNA into its own separate well containing working solution and mix well by pipetting up and down.

8. Incubate the microplate at room temperature for 1-5 minutes in the dark.

9. Measure fluorescence using a microplate reader to set to 485 nm excitation/530 nm emission maxima or other filter combination for detecting green fluorescence (e.g., FITC filter set).

10. Generate a standard curve to determine the unknown DNA concentration (see Figure 2). Average the triplicate values for each sample and subtract the average zero DNA value from each data point. Plot the fluorescence values for the DNA standards on the y-axis and ng/well DNA on the x-axis, and fit a trend line through these points to generate a standard curve with a y-intercept = 0. Use the equation for the standard curve trend line to calculate the amount of unknown DNA in each well (y = fluorescence and x = ng DNA per well). Note: the standard curve shown in Figure 2 is for reference only. You must generate your own standard curve using your instrument to calculate the amount of DNA in your unknown samples.

Continued next page

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 AccuBlue High Sensitivity reagent is linear from 0.2 – 100 ng dsDNA. The dynamic range can be extended to 200 ng with some loss of linearity.

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) pipette accuracy, and (5) microplate manufacturers.

The effects of common DNA contaminants such as salts, solvents, detergents and protein on the AccuBlue High Sensitivity assay are listed in Table 1. Please also see our AccuClear Ultra High Sensitivity dsDNA Quantitation Assays (related products), which have different tolerances for certain contaminants compared to AccuBlue High Sensitivity.

Table 1. Effect of common DNA contaminants on AccuBlue assay signal

Compound	Initial concentration in DNA sample	Final concentration in assay (200 uL)	Result
Ammonium Acetate	100 mM	5 mM	Pass
Sodium Acetate	600 mM	30 mM	Pass
Sodium Chloride	200 mM	10 mM	Pass
Magnesium Chloride	25 mM	1.25 mM	Pass
Phenol	2 %	0.1 %	Pass
Ethanol	10 %	0.5 %	Pass
Chloroform	2 %	0.1 %	Pass
Sodium Dodecyl Sulfate	0.2 %	0.01 %	Pass
Triton X-100	0.2 %	0.01 %	Pass
Bovine Serum Albumin	200 mg/mL	10 mg/mL	Pass*
dNTPs**	2 mM	100 uM	Pass
Polyethylene Glycol	40%	2 %	Pass
Agarose	2%	0.1 %	Pass

Triplicate DNA standard curves were assayed in the presence or absence of the contaminants at the indicated final concentrations. Pass indicates that there was < 20% change in signal 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. *Pass with some perturbation of standard curve linearity. ** Mix of dATP, dCTP, dGTP, dTTP.

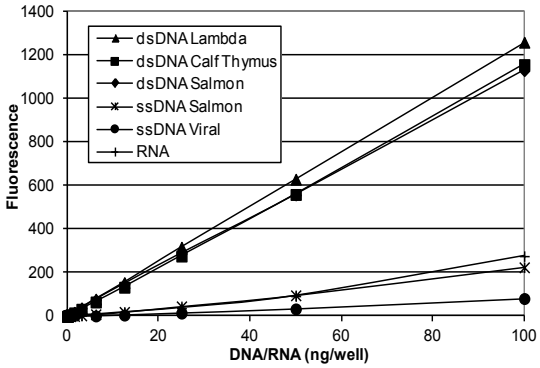

Figure 2: AccuBlue High Sensitivity dsDNA Quantitation kit selectivity and sensitivity for dsDNA. Triplicate samples of dsDNA or ssDNA from various sources or mouse liver RNA were assayed using AccuBlue and read at 485/530nm.

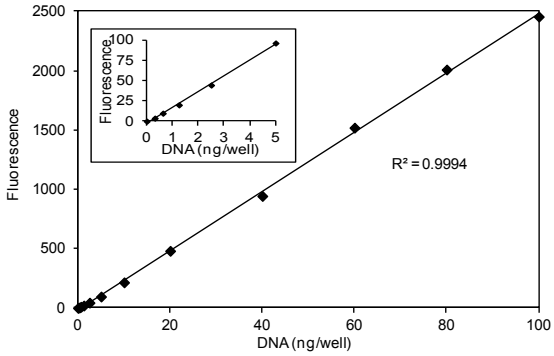

Figure 3: Example of AccuBlue High Sensitivity dsDNA standard curve.

Related Products

Catalog number	Product
E90001	AccuLite™470 Mini Fluorometer
31006C	AccuBlue™ High Sensitivity dsDNA Standards, Set of 7
31006	AccuBlue™ High Sensitivity dsDNA Quantitation Kit with 8 DNA Standards (1000 assays)
31028	AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit with 7 DNA Standards (1000 assays)
31029	AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit with 1 DNA Standard (2000 assays)
31027	AccuClear™ Ultra High Sensitivity dsDNA Quantitation Solution (1000 assays)
31007	AccuBlue™ Broad Range dsDNA Quantitation Kit with 9 DNA Standards (1000 assays)
31009-T	AccuBlue™ Broad Range dsDNA Quantitation Solution, trial size
41003	GelRed™ Nucleic Acid Gel Stain, 10,000X in water
31003-T	Fast EvaGreen® qPCR Master Mix, trial size
31020-T	Fast Plus EvaGreen® qPCR Master Mix, trial size

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF™dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

Appendix: AccuBlue High Sensitivity Assay Protocol for the AccuLite 470 Fluorometer

Sample Preparation

Note: if using Mini Glass Tubes, 100 uL sample volume can be used. Scale all volumes in the reaction (working solution and DNA) proportionally.

1. Prepare working solution as described in the AccuBlue High Sensitivity protocol.
2. For each sample to be tested, pipette 200 uL of the working solution into a 0.2 mL thin-walled clear PCR tube. To test samples in triplicate, prepare three tubes for each sample. Prepare two additional tubes for standards.
3. Prepare standards. Only the 0 ng DNA standard (blank) and 100 ng DNA standard are required. Pipette 10 uL of the 0 ng DNA standard into the 0 ng DNA tube (blank). Pipette 10 uL of the 10 ng/uL DNA standard into the 100 ng DNA tube. Pipette up and down or vortex to mix.
4. Prepare samples by pipetting 10 uL of each sample DNA per tube. Pipette up and down or vortex to mix.

Calibration

To move to a previous screen at any time, select Return. Continue selecting Return to go back to the Main Menu.

1. From the AccuLite Main Menu, select Calibrate.
2. Select Accu dsDNA from the assay list.
3. Insert the blank tube and close the cover. Select Blank.
4. The standard value 00100.000 will display. Insert the 100 ng DNA standard tube and close the cover. Press Measure.
5. Calibration Finished will appear on the screen.
6. Select Return to return back to the Main Menu.

Sample Measurement

1. From the AccuLite Main Menu, select Measure.
2. Select Accu dsDNA from the assay list.
3. Insert the first sample tube and close the cover. Select Measure. The value shown is ng DNA per tube.
4. Select Save to save the data in the meter.

   Alternatively, you can manually the record data without saving, then select Return.
5. Insert next sample and select Measure.
6. After reading all samples, select Return repeatedly to navigate back to main menu.

Retrieving Saved Data

1. From the AccuLite Main Menu, select Data.
2. Select Accu dsDNA from the assay list.
3. Use the arrow keys to navigate through saved data points. Data points are numbered (##) in order of measurement.
4. To erase data, select Erase All and Confirm.
5. To return to previous screens, select Return.

Performing a Full Calibration Curve with AccuLite

The first time you perform the assay, or if unexpected results are obtained, you may wish to perform a full calibration curve to verify that the assay is performing properly. In this case, perform the 2 point calibration as described above, then read the full set of standards as if they were unknown samples. Plot the standard curve as described in the AccuBlue High Sensitivity protocol.

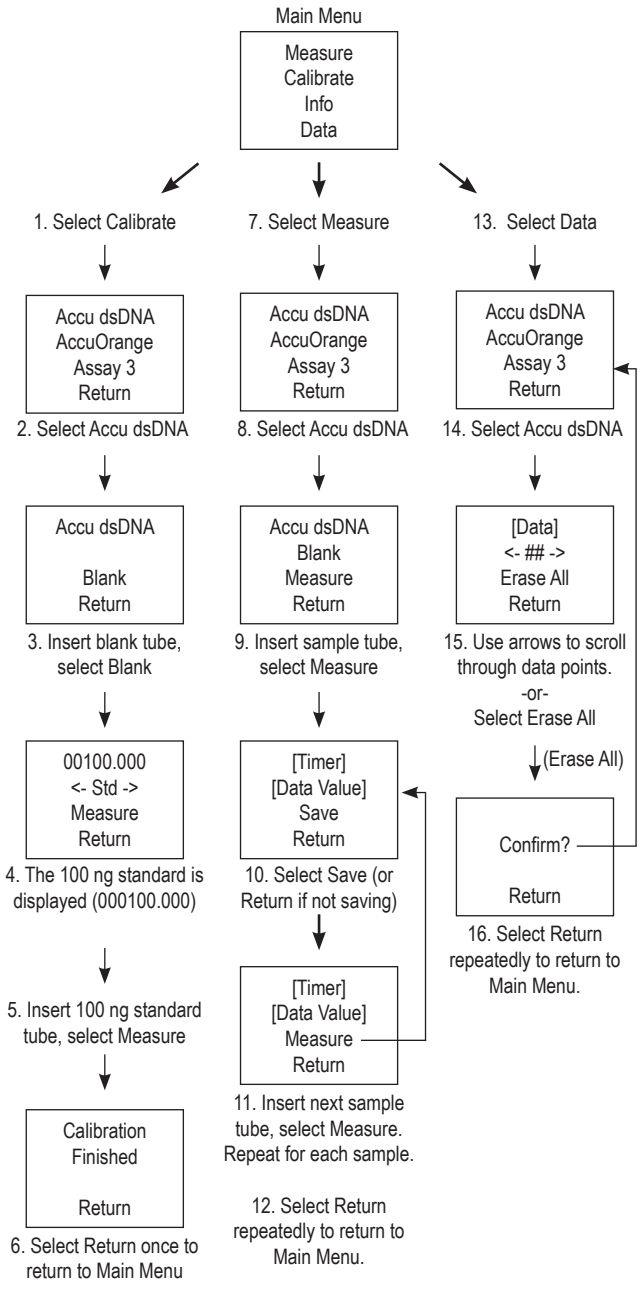

Figure 4. AccuLite user menu tree showing AccuBlue High Sensitivity calibration, measurement, and data retrieval steps. See the AccuLite user manual for complete user menu tree.