Revised: Aug 19, 2015

Product Information

AccuBlue™ Broad Range dsDNA Quantitation Kits with 9 DNA Standards

Catalog Number:

31007-T AccuBlue™ Broad Range dsDNA Quantitation Kit with DNA Standard, Trial Size
31007 AccuBlue™ Broad Range dsDNA Quantitation Kits with 9 DNA Standards

Kit Contents

Component	31007-T 200 assays	31007 1000 assays
AccuBlue Broad Range Buffer	50 mL 99975	250 mL 99973
AccuBlue Broad Range Dye (100X in DMSO)	1 mL 99974	3 X 1mL 99974
AccuBlue Broad Range Enhancer (100X in water)	1 mL 99943	3 X 1 mL 99943
dsDNA Standard in 10 mM Tris pH 7.5, 1 mM EDTA, 2 mM sodium azide 200 ng/uL dsDNA from calf thymus	0.5 mL 31007C-T	N/A
dsDNA Standards in 10 mM Tris pH 7.5, 1 mM EDTA, 2 mM sodium azide 0, 2, 6.25, 12.5, 25, 50, 100,150, and 200 ng/uL dsDNA from calf thymus	N/A	Set of 9, 0.5 mL each 31007C

Storage and Handling

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

Spectral Properties

Ex/Em 350/460nm (in the presence of dsDNA). See Figure 1 for spectra.

Product Description

The AccuBlue™ Broad Range Quantitation Kit is designed for quantitation of dsDNA in the range of 2 to 2000 ng in a 200 uL assay volume. Unlike absorbance-based measurements, the assay is selective for dsDNA over ssDNA or RNA (Figure 2). The quantitation assay offers the advantages of a wide dynamic range and high sensitivity over other traditional methods of DNA quantitation. The kit contains the AccuBlue Broad Range dsDNA Quantitation Buffer, Dye solution, Enhancer solution, and pre-diluted dsDNA Standards. The assay kit is tolerable to common contaminants such as proteins, salts, organic solvents and detergents. See Table 1 for more information. AccuBlue Broad Range dsDNA Quantitation Solution (cat. no. 31009) is available separately for customers who wish to use their own DNA standard.

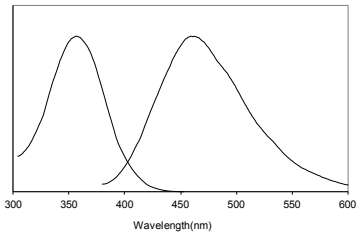

Figure 1: Excitation and emission spectra for AccuBlue Broad Range dsDNA quantitation reagent in the presence of excess dsDNA.

Assay Protocol

Note: see the Appendix for information on using the AccuBlue Broad Range assay with the AccuLite™ 350 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. Remove the DNA quantitation kit from storage and warm all components to room temperature before use. Accublue Broad Range dye is provided in DMSO, which may freeze during storage at 4°C. You can warm up all kit components in a 37°C water bath; be sure to allow solutions to equilibrate to room temperature before using. 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.

3. For 31007, use the DNA standards provided (31007C). For 31007-T (trial size kit), prepare a set of DNA standards by serial dilution of the 200 ng/uL standard provided in TE buffer (10 mM Tris pH 7-8, 1 mM EDTA) as shown in Table 1 below. The DNA standards can be stored at 4°C for at least 6 months if sodium azide is added to the TE buffer used for dilution at a final concentration of 2 mM.

Table 1. Preparation of DNA standards for 31007-T (trial size kit)

Standard	DNA	TE
200 ng/uL	100 uL of 200 ng/uL standard	None
150 ng/uL	75 uL of 200 ng/uL standard	25 uL
100 ng/uL	100 uL of 200 ng/uL standard	100 uL
50 ng/uL	100 uL of 100 ng/uL standard	100 uL
25 ng/uL	100 uL of 50 ng/uL standard	100 uL
12.5 ng/uL	100 uL of 25 ng/uL standard	100 uL
6.25 ng/uL	100 uL of 12.5 ng/uL standard	100 uL
2 ng/uL	32 uL of 6.25 ng/uL standard	68 uL
0 ng/uL	None	100 uL

4. Prepare 200 uL of working solution for each sample to be tested. Working solution should be used within an hour after preparation for best results, but it can be stored and used up to 24 hours later, with only minor loss of accuracy. During storage dye precipitation may occur, but can be resuspended by vortexing. 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. Combine reagents as shown in Table 2 below and mix well by vortexing or shaking.

Table 2: Preparation of 20 mL AccuBlue Broad Range working solution (scale volumes to prepare the amount required for assay).

Component	Volume
AccuBlue Broad Range Buffer	20 mL
AccuBlue Broad Range Dye (100X)	200 uL
AccuBlue Broad Range Enhancer (100X)	200 uL

5. For each sample, pipette 200 uL of the AccuBlue 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.

Continued next page

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

7. Incubate the microplate at room temperature for 10 minutes in the dark. The assay plate should be read within 1 hour for best results, but can be stored and read up to 24 hours after preparation with only minor loss of accuracy.

8. Measure fluorescence using a microplate reader with 350 nm excitation/460 nm emission.

9. Generate a standard curve to determine the unknown DNA concentration (see Figure 3). 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 3 is for reference only. You must generate your own standard curve using your instrument to calculate the amount of DNA in your unknown samples.

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 Broad Range assay is linear from 2 – 2000 ng dsDNA. The dynamic range can be extended to 4000 ng with some loss of linearity (Figure 4). If lower end standards are desired, you can further dilute any of the standards with 1X TE to a concentration of 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) pipette accuracy, and (5) microplate manufacturers.

The effects of common DNA contaminants such as salts, solvents, detergents and protein on the AccuBlue Broad Range assay are listed in Table 3. Please also see our AccuBlue High Sensitivity and AccuClear™ Ultra High Sensitivity dsDNA Quantitation Assays (related products), which have different tolerances for certain contaminants compared to AccuBlue Broad Range.

Table 3. 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 Chloride	1 M	50 mM	Pass
Ethanol	0.5 %	10 %	Pass
Phenol	0.1 %	2 %	Pass
Sodium Dodecyl Sulfate	0.01 %	0.2 %	Pass
Triton X-100	0.01 %	0.2 %	Pass
Bovine Serum Albumin	1 mg/mL	20 mg/mL	Pass*
dNTPs**	100 uM	2 mM	Pass

Triplicate samples of 2000 ng dsDNA 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 350 nm and fluorescence intensity was measured at 460 nm on a Molecular Devices Gemini XS microplate reader. *Pass with some perturbation of standard curve linearity. ** Mix of dATP, dCTP, dGTP, dTTP.

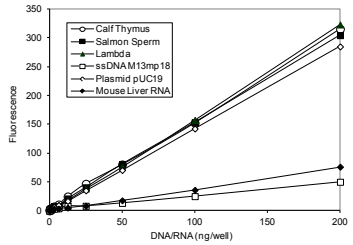

Figure 2. Relative fluorescence intensities of different nucleic acids using the AccuBlue Broad Range dsDNA Quantitation kit.

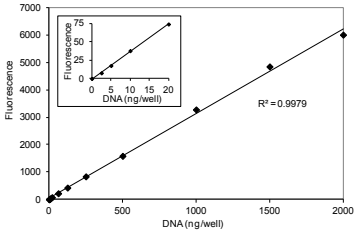

Figure 3: Standard curve of calf thymus DNA assayed using AccuBlue Broad Range Kit and read on a microplate reader (Ex/Em 350/460). Inset shows the lower end of the titration.

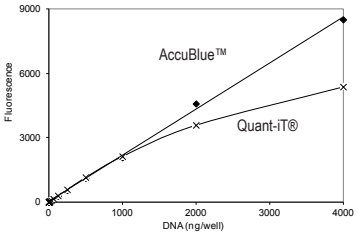

Figure 4: Two-fold dilutions of calf thymus DNA were assayed using AccuBlue or Quant-iT® Broad Range assay kits. AccuBlue has improved linearity and wider dynamic range than the Quant-iT Broad Range.

Related Products

Catalog number	Product
E90000	AccuLite™350 Mini Fluorometer
31007C	AccuBlue™ Broad Range dsDNA Standrds, Set of 9
31009	AccuBlue™ Broad Range dsDNA Quantitation Solution
31006	AccuBlue™ High Sensitivity dsDNA Quantitation Kit with 8 DNA Standards
31028	AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit with 7 DNA Standards
31027	AccuClear™ Ultra High Sensitivity dsDNA Quantitation Solution
31006C	High sensitivity dsDNA standards, set of eight, 0.5 mL each
31007C	Broad range dsDNA standards, set of nine, 0.5 mL each
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 Broad Range Assay Protocol for the AccuLite™ 350 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 Broad Range 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 1000 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 100 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 AccuBlue BR from the assay list.
3. Insert the blank tube and close the cover. Select Blank.
4. The screen will display 01000.000. Insert the 1000 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 AccuBlue BR 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 AccuBlue BR 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 Broad Range protocol.

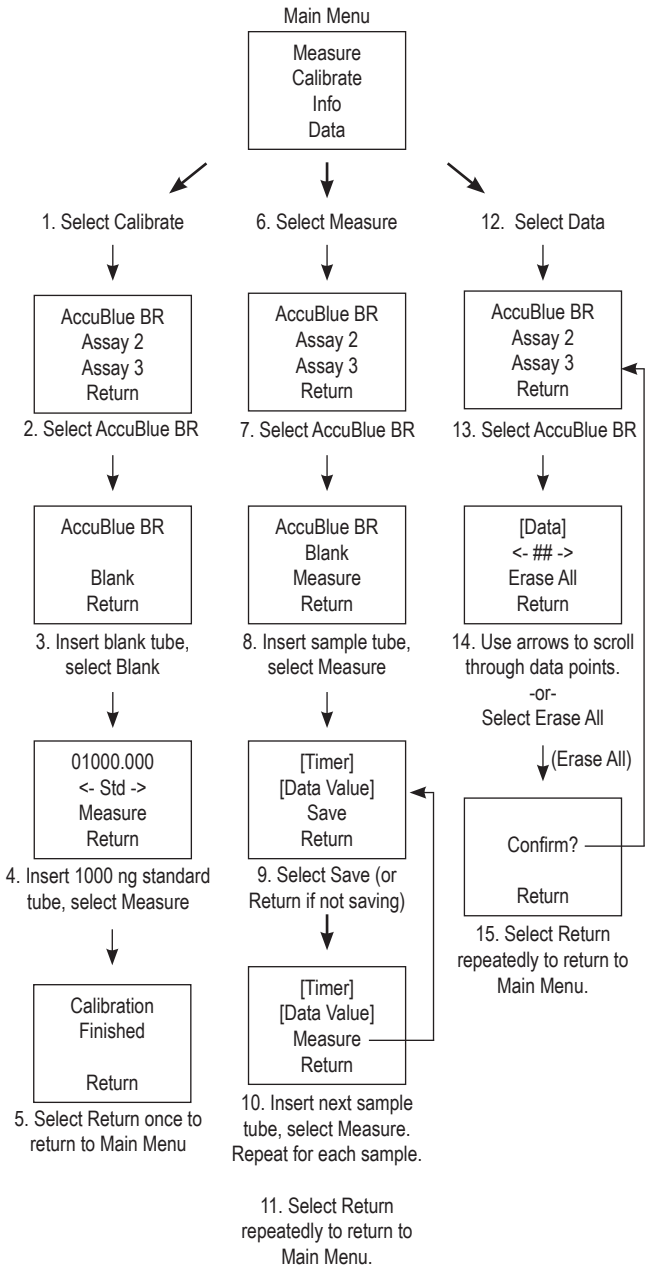

Figure 4. AccuLite user menu tree showing AccuBlue Broad Range calibration, measurement, and data retrieval steps. See the AccuLite user manual for complete user menu tree.