

AccuBlue™ High Sensitivity dsDNA Assay

Introduction

AccuBlue™ High Sensitivity dsDNA reagent is a fluorescent nucleic acid stain for quantitating minute amounts of double-stranded DNA. In conjunction with the micro-volume Thermo Scientific NanoDrop 3300 Fluorospectrometer, the AccuBlue HS dsDNA Assay provides a sensitive means of dsDNA quantitation with minimal consumption of sample. The main disadvantage of general UV spectroscopy for dsDNA quantitation is the contribution of signal from single-stranded DNA (ssDNA) and other contaminants, such as protein and extraction buffers. AccuBlue dsDNA HS reagent circumvents such contributions from interfering substances by exhibiting an emission maximum at 530 nm when bound to dsDNA (unbound AccuBlue HS reagent exhibits minimal fluorescence in solution). The sample retention technology of the NanoDrop™ 3300 allows significantly scaled-down reaction volumes, thereby using only a fraction of sample commonly needed for conventional cuvette-based fluorometers or plate readers. The NanoDrop 3300 has demonstrated a linear dynamic range for the AccuBlue dsDNA HS assay of 2ng/mL to 100 ng/mL. Measurements taken at the detection limit consume a total sample mass of 2 picograms of dsDNA.

Supplies

Equipment:

- NanoDrop 3300 Fluorospectrometer
- 2uL pipettor

Materials:

- Kimtech Science microfiber wipers (Cat # 7553)
- Nuclease free sterile amber or foil covered 1.5 mL polypropylene tubes
- 10 uL sterile nuclease free low retention tips

Reagents:

- AccuBlue™ High Sensitivity dsDNA Quantitation Kit (Biotium Cat# 31006)
- dsDNA standards (included in kit)

Create/Edit Method

1. Open the NanoDrop 3300 software and select **Create/Edit Method** button from the Main Menu.
2. Select Create Method to define the method. Select **Blue** as the LED source and click **Next**.
3. Select the following method parameters and click **Next** when done:
 - # of wavelengths = 1
 - lambda 1 = 530 nm,
 - Std Curve Type = Linear
 - Analysis Type = 1 pt.
 - Default units = ng/mL or pg/uL
4. Enter the Method name as AccuBlue HS dsDNA, and select **Nucleic Acid** as Type.
5. Enter 500 and 700, respectively or Graph Min. nm and Graph Max. nm. Click **Finished**.
6. Review information included in the Method List and use the **Edit Selected** button to make any corrections.
7. Select **Save** to save the method and **Exit** to exit the Method Editor and return to the main menu.

Standards and Sample Preparation

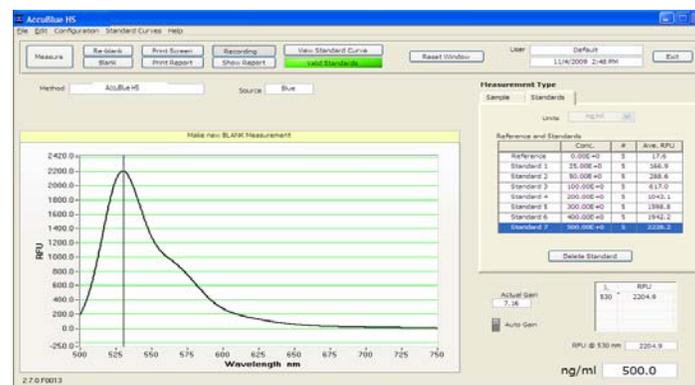
1. Allow the AccuBlue HS dsDNA kit and unknown dsDNA samples to warm to room temperature, then mix each component well.
2. Prepare the AccuBlue HS dsDNA working solution immediately before use by transferring 10 uL of the 100X AccuBlue Enhancer and 990 uL of the AccuBlue dsDNA HS Quantitation Solution to a 1.5mL pre labeled amber snap cap tube and mix well. Prepare only what you plan to use as precipitation may occur over time.
3. Add 20 uL of the AccuBlue HS dsDNA working solution into each tube. Amber tubes are recommended.
4. Add 1 uL of each of the pre-diluted dsDNA standards into correspondingly labeled tubes and mix well by pipetting up and down.
5. Add 1 ul of each of the unknown samples into correspondingly labeled tubes and mix by pipetting up and down.
6. Incubate all standards and unknown samples for 15 min at room temperature.

Procedure

- Before starting the procedure, clean the pedestals by applying 2 uL of nuclease free deionized water to the lower pedestal. Lower the upper arm to allow the water to come in contact with both measurement surfaces.
- Raise the upper arm and firmly blot the two pedestals with a dry lab wipe. Make sure there are no traces of lint on the measurement surfaces before continuing
- Open the operating software and click on the **Nucleic Acid Quantitation** button. Select **AccuBlue HS dsDNA** from the Select method window
- Add 2 uL of quantitation solution (no dye, no sample) to the lower pedestal. Lower the arm and click **F3** or the **Blank** button.
- When the measurement is complete, lift the arm and use a dry laboratory wipe to blot the buffer from both the bottom and upper measurement surfaces.
- To verify a proper baseline, apply a fresh aliquot of quantitation solution to the lower pedestal, lower the arm and click **F1** or **Measure**.
- Under Measurement type, click on the **Standards** tab. Select **Standard 1**, enter a value and click **OK**. Continue entering the values from lowest to highest for each standard to be measured. (See example data below)
- In the Reference and Standards table, highlight the Reference standard. Briefly mix the reference solution (containing quantitation solution and AccuBlue dsDNA HS working solution only) and transfer 2 uL of the solution onto the lower pedestal. Lower the arm and click **F1** or the **Measure** button. A pop up window will ask for confirmation of the units; the recommended units are ng/mL or pg/uL. Confirm units and select **OK**.
- Measure up to 5 replicates of the reference solution using a fresh 2 uL aliquot for each measurement.
- Mix each standard solution briefly prior to measurement and transfer 2 uL onto the lower pedestal. Lower the arm and click **F1** or the **Measure** button.
- Measure up to 5 replicates of each standard using a fresh 2 uL aliquot for each measurement.
- Once all standards have been measured, select the Standard Curve Type (Interpolation, Linear, 2° polynomial, 3° polynomial) that best fits the standards data set.
- Click on the **Sample** tab under Measurement Type, and enter the unknown samples' respective ID information. If a dilution of the unknown sample was made, enter the dilution factor in the box below the sample ID window.
- Add 2 uL of the sample and use the **F1** key or click the **Measure** button to initiate the measurement cycle. Use a fresh aliquot of sample for each measurement.

dsDNA [ng/ml] in assay	Avg RFU n=5	SD RFU	% CV
0	18	1.0	5.8
25	173	6.5	3.9
50	287	2.4	0.8
100	617	1.7	0.3
200	1043	30	2.9
300	1599	73	4.6
400	1942	55	2.8
500	2228	38	1.7

Example Data for AccuBlue HS ds DNA assay



Example spectrum for AccuBlue HS dsDNA sample

For technical questions, contact Technical Support at 302-479-7707 or send an email to info@nanodrop.com.
Outside of the US or Canada, contact your local distributor.

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