Helixyte™ Green Fluorimetric dsDNA Quantitation Kit *Optimized for Broad Dynamic Range*

Catalog number: 17645, 17646
Unit size: 200 Tests, 1000 Tests

Component	Storage	Amount (Cat No. 17645)	Amount (Cat No. 17646)
Component A: Helixyte™ Green BR	Freeze (< -15 °C), Minimize light exposure	1 vial (0.1 mL-200X in DMSO)	1 vial (0.5 mL, 200X in DMSO)
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (50 mL)	3 bottles (85 mL/bottle)
Component C: DNA Standard	Refrigerated (2-8 °C)	1 vial (1 mL, Calf thymus DNA: 100 ug/mL)	1 vial (5 mL, Calf thymus DNA: 100 ug/mL)

OVERVIEW

DNA Quantitation is a very important task in DNA sample preparations for various analyses. Helixyte™ Green Fluorimetric dsDNA Quantitation Kit provides a rapid method to quantify dsDNA with Helixyte™ Green BR . The assay is linear over three orders of magnitude and is a few magnitudes more sensitive than UV absorbance readings. Helixyte™ Green BR exhibits large fluorescence enhancement upon binding to dsDNA and has little sequence dependence, allowing to the accurate measurement of DNA samples from various sources, including genomic DNA, viral DNA, miniprep DNA or PCR amplification products. The assay is highly selective for double-stranded DNA (dsDNA) over RNA and is optimized to measure DNA concentrations from 10 pg/µL to 10 ng/µL.

AT A GLANCE

Protocol Summary

1. Prepare dSDNA standards, test samples and dye working solution
2. Add DNA standards or test samples (50 uL)
3. Add Helixyte™ Green BR working solution (50 uL)
4. Incubate at room temperature for 2 minutes
5. Monitor fluorescence intensity at Ex/Em= 490/530 nm

Important Note

The following protocol is provided as an example for quantifying dsDNA with Helixyte™ Green BR. Warm all the components to room temperature before opening. No data are available for the mutagenicity or toxicity of Helixyte™ Green dsDNA stain. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	515 nm
Emission	530 nm
Excitation	490 nm
Recommended plate	Solid black

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/17645

dsDNA standard

Add 100 uL of 100 ug/mL dsDNA Standard Solution (Component C) to 400 uL Assay Buffer (Component B) to generate 20 ug/mL dsDNA standard solution. Then perform 1:3 serial dilutions by Assay Buffer (Component B) to get serially diluted dsDNA standards ranging from 0 to 20 ug/mL.

PREPARATION OF WORKING SOLUTION

Helixyte™ Green BR working solution

Add 50 uL Helixyte™ Green BR (Component A) into 5 mL of Assay Buffer (Component B) to make a total volume of 5.050 mL. Protect the working solution from light by covering it with foil or placing it in the dark.

Note: We recommend preparing this solution in a plastic container rather than glass, as the dye may adsorb to glass surfaces. For best results, this solution should be used within a few hours of its preparation.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of dsDNA standards and test samples in a clear bottom 96-well microplate. DS=dsDNA standards (DS1-DS7, 20 to 0.027 ug/mL); BL=Blank Control; TS=Test Samples

BL	BL	TS	TS
DS1	DS1	...	...
DS2	DS2	...	...
DS3	DS3		
DS4	DS4		
DS5	DS5		
DS6	DS6		
DS7	DS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
DS1-DS7	50 uL	Serial Dilutions (20 to 0.027 ug/mL)
BL	50 uL	Assay Buffer (Component B)
TS	50 uL	Test Sample

1. Prepare dsDNA standards (DS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2.

For a 384-well plate, use 25 uL of reagent per well instead of 50 uL.

Note: Treat cells or tissue samples as desired.

2. Add 50 uL of Helixyte™ Green BR dye working solution (2X) to each well of dsDNA standard, blank control, and test samples to make the total assay volume 100 uL/well. For a 384-well plate, add 25 uL ofHelixyte™ Green BR dye working solution into each well instead, for a total volume of 50 uL/well.
3. Incubate the reaction for 2 minutes at room temperature, protected from light.
4. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 490/530 nm (cut off at 515nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate DNA samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-onlinecalculator

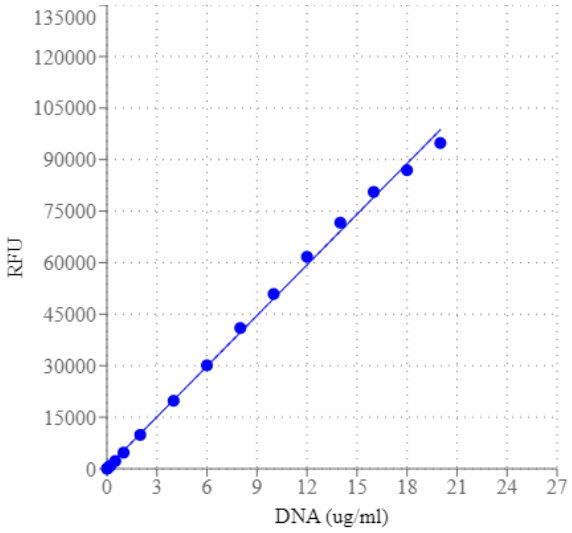

Figure 1. dsDNA dose response was measured with Helixyte™ Green Fluorimetric dsDNA Quantitation Kit in a 96-well solid black plate.

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