Helixyte™ Green Fluorimetric dsDNA Quantitation Kit *Optimized for Microplate Readers*

Catalog number: 17650
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 17650)
Component A: Helixyte Green™	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL, 200X in DMSO)
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (50 mL)
Component C: Calf thymus DNA Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (200 µL, 100 µg/mL)

OVERVIEW

Helixyte™ Green dsDNA Quantitation Assay Kit can be used for selectively detecting as little as 25 pg/ml of dsDNA in the presence of ssDNA, RNA, and free nucleotides. Helixyte™ Green exhibits large fluorescence enhancement upon binding to dsDNA. The assay is linear over three orders of magnitude and has little sequence dependence, allowing you to accurately measure DNA from many sources, including genomic DNA, viral DNA, miniprep DNA, or PCR amplification products. Helixyte™ Green dsDNA Quantitation Assay Kit is a few magnitudes more sensitive than UV absorbance readings. It is specific for dsDNA in the presence of equimolar amounts of RNA. The kit is robust with a mix and read format compatible with 96- and 384-well fluorescence-based microplate readers. It can also be used with a bench top fluorometer or a hand-held fluorescence meter (e.g., Qubit fluorometer).

AT A GLANCE

Protocol Summary

1. Add 100 µL dsDNA standards or test samples
2. Add 100 µL Helixyte Green™ working solution
3. Incubate at RT for 5-10 minutes
4. Monitor the fluorescence at Ex/Em=490/525 nm

Important Note

The following protocol is an example for quantifying dsDNA with Helixyte Green™. Allow all the components to warm to room temperature before opening. No data are available addressing 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.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/17650

dsDNA standard

Add 2 µL of 100 µg/mL dsDNA stock solution (Component C) to 198 µL of Assay buffer (Component B) to have 1 µg/mL dsDNA solution, and then perform 1:2 serial dilutions to get serially diluted dsDNA standard (DS7 - DS1). For a lower range of DNA, further dilutions can be performed to achieve as low as 25 ng/mL concentration.

PREPARATION OF WORKING SOLUTION

Prepare Helixyte Green™ working solution by adding 50 µL of Helixyte Green™ (Component A) into 10 mL of Assay Buffer (Component B). 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 solid black 96-well microplate. DS= dsDNA Standards (DS1 - DS7, 15.6 to 1000 ng/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	100 µL	Serial Dilutions (15.6 to 1000 ng/mL)
BL	100 µL	TE
TS	100 µL	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 µL of reagent per well instead of 100 µL.
2. Add 100 µL of Helixyte Green™ working solution to each well of dsDNA standard, blank control, and test samples to make the total dsDNA assay volume of 200 µL/well. For a 384-well plate, add 25 µL of BLANK assay mixture into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction at room temperature for 5 to 10 minutes, protected from light.
4. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 490/525 nm (cut off at 515 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

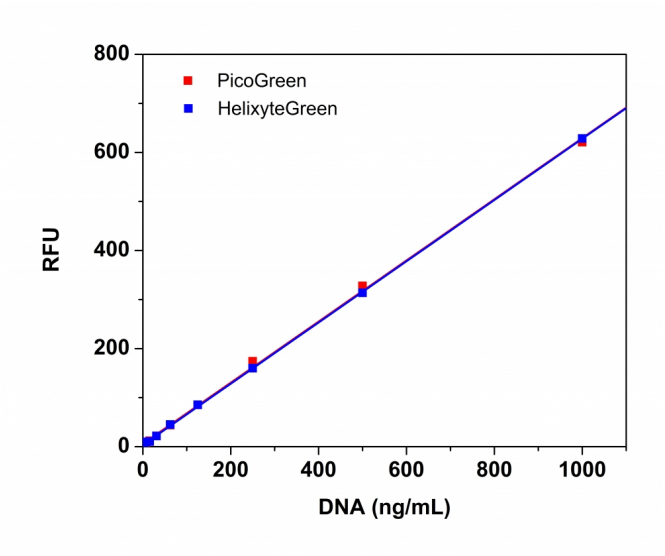

Figure 1. Comparison of dsDNA dose response using the Helixyte Green™ (blue) with Invitrogen™ Quant-iT™ PicoGreen® dsDNA Reagent (red). dsNDA standards were incubated in cuvettes and measured using varian cary eclipse fluorescence spectrophotometer.

DISCLAIMER

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