Helixyte™ Green Fluorimetric dsDNA Quantitation Kit *High Sensitivity*

Catalog number: 17651
Unit size: 1000 Tests

Component	Storage	Amount (Cat No. 17651)
Component A: Helixyte Green™	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mL, 200X in DMSO)
Component B: 20X Assay Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (25 mL)
Component C: Calf thymus DNA Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mL, 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. It can be used with a bench top fluorometer or a hand-held fluorescence meter (e.g., Qubit fluorometer). This kit is an excellent replacement for Quant-iT™ PicoGreen® dsDNA Assay Kit (Quant-iT™ and PicoGreen® are the trademarks of Invitrogen).

AT A GLANCE

Protocol Summary

1. Add 1mL of dsDNA standards or test samples to each cuvette.
2. Add 1 mL of the Helixyte Green™ working solution.
3. Incubate at room temperature for 5 to 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 components to warm to room temperature before opening. There is no available data on the mutagenicity or toxicity of Helixyte Green™ dsDNA stain. However, since this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care. Handle the DMSO stock solution with particular caution, as DMSO is known to facilitate the entry of organic molecules into tissues.

KEY PARAMETERS

Spectrofluorometer

Emission	525 nm
Excitation	490 nm
Cutoff	515 nm

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Assay Buffer (1X)

1. Prepare a 1X Assay buffer by diluting the concentrated buffer 20-fold with sterile, distilled, DNase-free water.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/17651

dsDNA standard

For high range standard curve:Add 30 µL of 100 µg/mL dsDNA stock solution (Component C) to 1.47 mL of 1X Assay buffer to have 2000 ng/mL dsDNA solution, and then perform 1:2 and 1:10 serial dilutions to get 1000, 100, 10, 1 and 0 ng/mL.For low range standard curve:Add 40 µL of 2 µg/mL dsDNA stock solution to 1.56 mL of 1X Assay buffer to have 50 ng/mL dsDNA solution, and then perform 1:2 and 1:10 serial dilutions to get 25, 2.5, 0.25, 0.025 and 0 ng/mL.

PREPARATION OF WORKING SOLUTION

Helixyte Green™ Working Solution

1. To prepare the Helixyte Green™ working solution, make a 200-fold dilution of the concentrated DMSO solution in 1X assay buffer. For example, to prepare enough working solution to assay 10 samples in a final volume of 2 mL each, add 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 a dark location.

   Note: We recommend using a plastic container to prepare this solution, as the dye can adhere to glass surfaces. For optimal performance, use the solution within a few hours of preparation.

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 1 mL of Helixyte Green™ working solution to each cuvette containing 1 mL of the dsDNA standard, blank control, and test samples. This will bring the total volume of the dsDNA assay in each cuvette to 2 mL.
2. Incubate the reaction at room temperature for 5-10 minutes, keeping it protected from light.
3. Monitor the fluorescence increase with a spectrofluorometer at Ex/Em = 490/525 nm, Cutoff = 515 nm.

   Note: To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.

EXAMPLE DATA ANALYSIS AND FIGURES

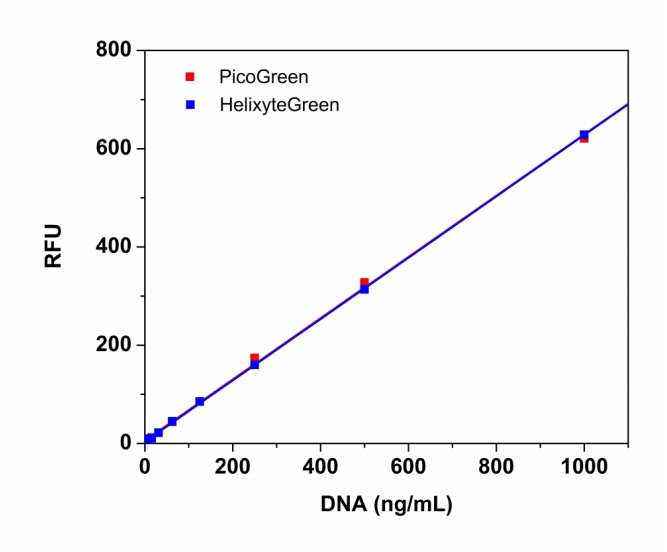

Figure 1. Comparison of dsDNA dose response using the Helixyte Green™ (blue) with Invitrogen™ Quant-iT™ PicoGreen® dsDNA Reagent (red). dsNDA standards were incubated in cuvettes and measured using varian cary eclipse fluorescence spectrophotometer.

DISCLAIMER

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