Helixyte™ Green Fluorimetric dsDNA Quantitation Kit *High Sensitivity*

Catalog number: 17651
Unit size: 200 Tests

Component	Storage	Amount
Component A: Helixyte Green™	Freeze (<-15 °C), Minimize light exposure	1 mL (200X in DMSO)
Component B: 20X Assay Buffer	Freeze (<-15 °C), Minimize light exposure	25 mL
Component C: Calf thymus DNA Standard	Freeze (<-15 °C), Minimize light exposure	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 dsDNA standards or test samples to each cuvette
2. Add 1mL Helixyte Green™ working solution
3. Incubate at RT for 5-10 minutes
4. Monitor the fluorescence at Ex/Em=490/525 nm

Important 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 is 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.

KEY PARAMETERS

Instrument:	Spectrofluorometer
Excitation:	490 nm
Emission:	525 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.

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

PREPARATION OF STANDARD SOLUTION

dsDNA standard

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

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

Prepare Helixyte Green™ working solution by making 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 2 mL final volume, 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 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

1. Add 1 mL of Helixyte Green™ working solution to each cuvette containing 1 mL of the dsDNA standard, blank control, and test samples to make the total dsDNA assay volume of 2 mL/cuvette.

2. Incubate the reaction at room temperature for 5 to 10 minutes, protected from light.

3. Monitor the fluorescence increase with a spectroflurometer at Ex/Em = 490/525 nm.

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

EXAMPLE DATA ANALYSIS AND FIGURES

Example data analysis and images of this product can be found on the web at: https://www.aatbio.com/products/helixyte-green-fluorimetric-dsdna-quantitation-kit-high-sensitivity

DISCLAIMER