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

Catalog number: 17623
Unit size: 200 Tests

Component	Storage	Amount
Component A: Helixyte™ Green ssDNA	Freeze (< -15 °C), Minimize light exposure	1 vial (200 µL)
Component B: Assay Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (50 mL)
Component C: ssDNA Standard	Refrigerated (2-8 °C), Minimize light exposure	30 µL (100 µg/mL)

OVERVIEW

Helixyte™ Green Fluorimetric ssDNA Quantitation Kit is designed to measure single-stranded DNA in an easy and accurate format. The kit has all the essential reagents, including Helixyte™ Green ssDNA reagent, dilution buffer, and prediluted DNA standards. Helixyte™ Green ssDNA reagent is a sensitive fluorescent nucleic acid probe for quantifying oligonucleotides and single-stranded DNA (ssDNA) in solution. It enables researchers to quantify as little as 100 pg/mL oligonucleotide or ssDNA. This sensitivity exceeds that achieved with absorbance methods by more than 10,000-fold. With this kit, as little as 1 ng/mL oligonucleotide or ssDNA might be detected. A few ssDNAs were quantified with this kit, including M13 and ϕX174 viral DNA and denatured calf thymus DNA that had similar sensitivity. Their detection limits were not significantly interfered by the common contaminants in nucleic acid preparations, including salts, urea, ethanol, chloroform, detergents, proteins, ATP, nucleotides and short oligonucleotides of six bases. However, double-stranded DNA (dsDNA) and RNA do interfere with the assay as Helixyte™ Green ssDNA reagent binds to dsDNA and RNA to generate additional fluorescence signal. Helixyte™ Green Fluorimetric ssDNA Quantitation Kit is optimized for quantifying dsDNA with a fluorescence microplate reader.

AT A GLANCE

Protocol summary

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

Important

The following protocol is an example of quantifying the ssDNA using Helixyte™ Green ssDNA. Allow all the components to warm to room temperature before opening. No data are available on the mutagenicity or toxicity of Helixyte™ Green ssDNA 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

Excitation	490 nm
Emission	525 nm
Cutoff	515 nm
Recommended plate	Solid black

PREPARATION OF STANDARD SOLUTION

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

ssDNA Standard solution

Add 10 uL of 100 ug/mL ssDNA Standard solution (Component C) to 190 uL of Assay Buffer (Component B) to get a 5 ug/mL standards solution, then perform 1:3 dilutions to obtain serially diluted ssDNA standards (SS2-SS7).

PREPARATION OF WORKING SOLUTION

Helixyte™ Green ssDNA working solution

Prepare the Helixyte™ Green ssDNA working solution by adding 100 µL of Helixyte™ Green ssDNA (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 It's recommended to prepare the working solution in a plastic container rather than a glass container, as the dye may adsorb to the glass surface. For best results, this solution should be used within a few hours after the dilution.

Note 10 mL of working solution is enough for one 96-well plate.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. The layout of ssDNA Standards and test samples in a solid black 96-well microplate. SS= ssDNA Standards (SS1 - SS7, 1667 to 2.3 ng/mL); BL=Blank Control; TS=Test Samples

BL	BL	TS	TS
SS1	SS1	…	…
SS2	SS2	…	…
SS3	SS3		
SS4	SS4		
SS5	SS5		
SS6	SS6		
SS7	SS7		

Table 2. The reagent composition for each well.

Well	Volume	Reagent
SS1-SS7	100 µL	Serial dilutions (1667 to 2.3 ng/mL)
BL	100 µL	Assay Buffer
TS	100 µL	Sample

1. Prepare ssDNA Standards (NS), 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 the Helixyte™ Green ssDNA working solution to each well of ssDNA Standards, blank control, and test samples to make the assay volume of 200 µL/well. For a 384-well plate, add 25 µL of the Helixyte™ Green ssDNA working solution into each well instead, to get 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

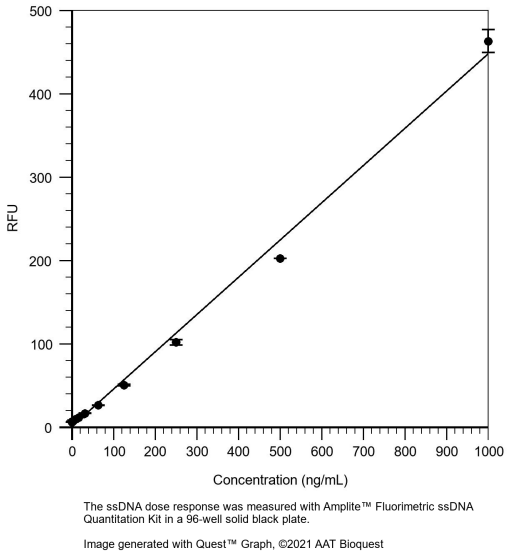

Figure 1. The ssDNA dose response was measured with Amplite™ Fluorimetric ssDNA Quantitation Kit in a 96-well solid black plate.

DISCLAIMER

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