Helixyte™ Green ssDNA reagent

Catalog number: 17620, 17621
Unit size: 1000 Tests, 10000 Tests

Component	Storage	Amount (Cat No. 17620)	Amount (Cat No. 17621)
Helixyte™ Green ssDNA reagent	Freeze (< -15 °C), Minimize light exposure	1000 Tests	10000 Tests

OVERVIEW

Synthetic oligonucleotides are used in a number of molecular biology techniques, such as DNA sequencing, site-directed mutagenesis, DNA amplification and in situ hybridization. The most commonly used technique for measuring oligonucleotide and ssDNA concentration is the determination of absorbance at 260 nm (A260). However, the absorbance method suffers great interferences resulted from various contaminants commonly found in nucleic acid preparations, including nucleotides, double-stranded nucleic acids and proteins. Helixyte™ Green ssDNA Quantifying Reagent is an excellent alternative for quantifying ssDNA and oligonucleotides with greatly improved sensitivity and selectivity. Helixyte Green ssDNA reagent is a positively charged fluorescent probe that binds to the hydrophobic pockets of ssDNA and forms a highly luminescent complex through the synergistic actions of stacking, hydrophobic forces, hydrogen bonding and electrostatic interactions. Helixyte Green ssDNA reagent has extremely low inherent fluorescence that is significantly enhanced upon binding to ssDNAs, resulting in a great enhancement in its fluorescence. It enables researchers to quantify as little as 100 pg/mL oligonucleotide or ssDNA (~500 pg/mL) with a standard spectrofluorometer and fluorescein excitation and emission wavelengths. This sensitivity exceeds the absorbance method by a few orders. As little as 1 ng/mL oligonucleotide or ssDNA can be detected with a fluorescence microplate reader. Helixyte Green ssDNA reagent has a large linear detection range of from 100 pg/mL to 1 µg/mL.

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/17620

ssDNA Standard solution

Add 10 uL of 100 ug/mL ssDNA Standard solution (Not provided) to 190 uL of buffer of your choice 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 reagent into 10 mL of buffer of your choice. 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.

Note 10 mM Tris-HCl (pH 8.0) with 0.1 mM EDTA can be used to make working solution and standards.

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	Buffer of your choice
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

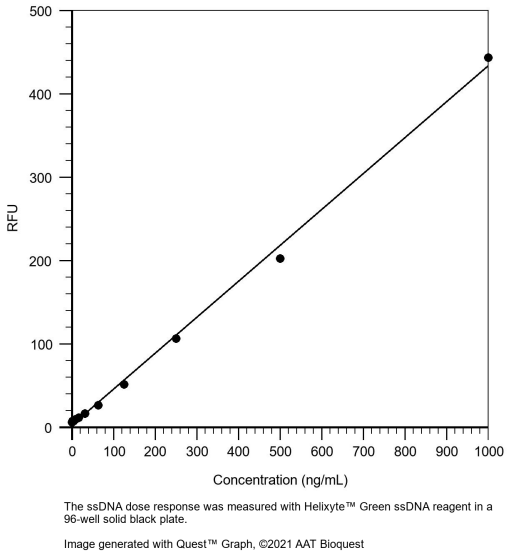

Figure 1. The ssDNA dose response was measured with Helixyte™ Green ssDNA reagent in a 96-well solid black plate.

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