Helixyte™ Green Fluorimetric Total Nucleic Acid Quantitation Kit *Optimized for Microplate Readers*

Catalog number: 17630
Unit size: 200 Tests

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

OVERVIEW

Helixyte™ Green Fluorimetric Total Nucleic Acid Quantitation Kit is designed to measure total amounts of nucleic acids, including double-stranded DNA (dsDNA), single-stranded DNA (ssDNA) and RNA in an easy and accurate format. The kit has all the essential reagents, including Helixyte™ Green All reagent, dilution buffer, and pre-diluted DNA standards. Helixyte™ Green All reagent is a sensitive fluorescent nucleic acid probe for measuring the total amounts of nucleic acids in a sample that may contain dsDNA, ssDNA, RNA and long oligonucleotides. Helixyte™ Green All reagent indiscriminately binds to dsDNA, ssDNA and RNA. Helixyte™ Green Fluorimetric Total Nucleic Acid Quantitation Kit is optimized for measuring the total amounts of nucleic acids with a fluorescence microplate reader.

AT A GLANCE

Protocol Summary

1. Add 100 µL of Nucleic Acid Standards or test samples
2. Add 100 µL of Helixyte™ Green All working solution
3. Incubate at room temperature 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 total nucleic acid content using Helixyte™ Green All. Allow all the components to warm to room temperature before opening. No data are available on the mutagenicity or toxicity of Helixyte™ Green All, the total nucleic acid 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/

Nucleic Acid Standard

Add 10 uL of 100 ug/mL Nucleic Acid Standard solution (Component C) to 190 uL of Assay Buffer (Component B) to get a 5 ug/mL standard solution and then perform 1:3 dilutions to obtain serially diluted Nucleic Acid Standard (NS1-NS7).

PREPARATION OF WORKING SOLUTION

Helixyte™ Green All working solution

Prepare the Helixyte™ Green All working solution by adding 100 µL of Helixyte™ Green All (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 the working solution is enough for one 96-well plate.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. The layout of Nucleic Acid Standards and test samples in a 96-well solid black microplate. NS= Nucleic Acid Standards (NS1 - NS7, 1667 to 2.3 ng/mL); BL=Blank Control; TS=Test Samples

BL	BL	TS	TS
NS1	NS1	…	…
NS2	NS2	…	…
NS3	NS3		
NS4	NS4		
NS5	NS5		
NS6	NS6		
NS7	NS7		

Table 2. The reagent composition for each well.

Well	Volume	Reagent
NS1-NS7	100 µL	Serial dilutions (1667 to 2.3 ng/mL)
BL	100 µL	Assay Buffer
TS	100 µL	Sample

1. Prepare Nucleic Acid 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 All working solution to each well of Nucleic Acid 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 All 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

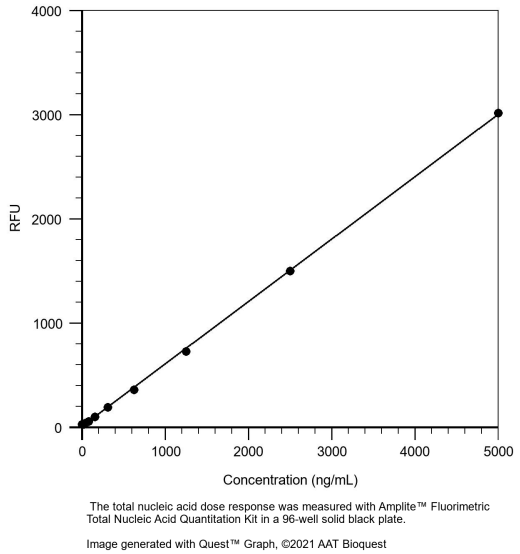

Figure 1. The total nucleic acid dose response was measured with Amplite™ Fluorimetric Total Nucleic Acid Quantitation Kit in a 96-well solid black plate.

DISCLAIMER

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