Helixyte™ Total DNA Quantification Assay Kit *4-2000 ng Broad Range*

Catalog number: 17633
Unit size: 500 Tests

Component	Storage	Amount (Cat No. 17633)
Component A: Helixyte™ Green DNA BR	Freeze (< -15 °C), Minimize light exposure	0.25 mL (100X in DMSO)
Component B: DNA Assay Buffer	Freeze (< -15 °C)	50 mL
Component C: Calf thymus DNA Standard	Freeze (< -15 °C), Minimize light exposure	1 mL (Calf thymus DNA: 100 ug/mL)

OVERVIEW

The Helixyte™ Total DNA Quantification Assay Kit offers a streamlined solution for accurately measuring DNA concentrations. With a pre-formulated, ready-to-use working solution, this kit simplifies workflows. Just prepare your sample, mix it with the Helixyte™ Green DNA BR reagent, and measure the DNA concentration using a fluorescence microplate reader.

This assay is optimized for high selectivity toward DNA over RNA, ensuring precise and reproducible quantification. It accommodates a wide range of DNA concentrations and reliably detects 4–2000 ng of DNA. The straightforward protocol involves diluting 1–50 µL of the sample into the provided 1X working solution and recording fluorescence intensity.

The kit is designed to tolerate common contaminants, including salts, free nucleotides, solvents, detergents, and proteins, minimizing interference and maximizing accuracy. Its highly specific formulation ensures superior detection of DNA over RNA and protein, making it ideal for a range of research and diagnostic applications requiring reliable and precise DNA quantitation.

AT A GLANCE

Protocol Summary

1. Add test samples (50 µL)
2. Add Helixyte™ Green DNA BR reagent working solution (50 µL)
3. Incubate at room temperature for 2 minutes
4. Monitor fluorescence intensity at Ex/Em = 490/530 nm

Important: The following protocol serves as an example for quantifying total DNA with Helixyte™ Green DNA BR. Ensure all components are brought to room temperature before opening. Please note that no data are available for the mutagenicity or toxicity of Helixyte™ Green DNA BR stain. As this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care. Exercise particular caution when handling the DMSO stock solution, as DMSO is known to facilitate the absorption of organic molecules into tissues.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	515 nm
Emission	530 nm
Excitation	490 nm
Recommended plate	Solid black

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/17633

DNA Standard
Add 80 µL of 100 µg/mL DNA Standard Solution (Component C) to 120 µL Assay Buffer (Component B) to generate 40 µg/mL DNA standard solution. Then perform 1:2 serial dilutions by Assay Buffer (Component B) to get serially diluted dsDNA standards ranging from 0 to 40 µg/mL.

PREPARATION OF WORKING SOLUTION

Helixyte™ Green DNA BR Working Solution (2X)

1. Add 50 µL of Helixyte™ Green DNA BR (Component A) to 2.5 mL of DNA Assay Buffer (Component B) and mix thoroughly. Protect the working solution from light by covering it with foil or storing 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 optimal results, use this solution within 2 hours of preparation.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of dsDNA standards and test samples in a solid black 96-well microplate. DS=DNA Standards (DS1-DS13, 40 to 0.0098 µg/mL); BL=Blank Control; TS=Test Samples.

BL	BL	DS8	DS8
DS1	DS1	DS9	DS9
DS2	DS2	DS10	DS10
DS3	DS3	DS11	DS11
DS4	DS4	DS12	DS12
DS5	DS5	DS13	DS13
DS6	DS6	TS	TS
DS7	DS7	TS	TS

Table 2. Reagent composition for each well.

Well	Volume	Reagent
DS1–DS7	50 µL	Serial Dilutions (40 to 0.0098 µg/mL)
BL	50 µL	Assay Buffer
TS	50 µL	Test Sample

1. Prepare DNA Standards (DS), 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 50 µL.

2. Add 50 µL of 2X working solution to each well containing DNA Standard, blank control, and test samples to achieve a total DNA assay volume of 100 µL/well. For a 384-well plate, add 25 µL of 2X working solution to each well, resulting in a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 2 minutes, protected from light.

4. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 490/530 nm (cut off at 515nm).

Data Analysis

For data analysis, we recommend using the Online Linear Regression Calculator available here. This tool facilitates linear, logarithmic, and semi-log regression analysis to help interpret your experimental results.

EXAMPLE DATA ANALYSIS AND FIGURES

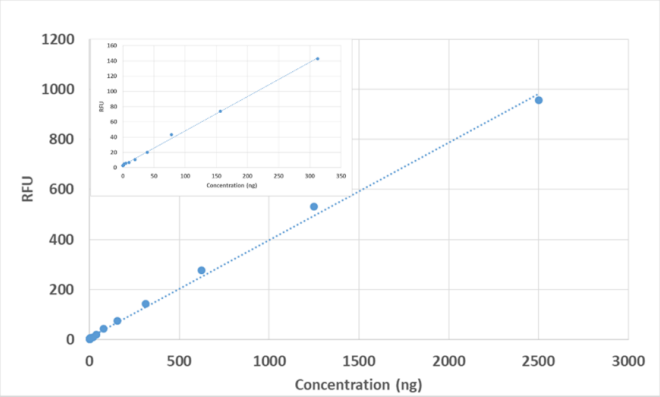

Figure 1. DNA dose response measured with Helixyte™ Total DNA Quantitation Assay Kit in a 96-well solid black plate.

DISCLAIMER

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