Helixyte™ Green dsDNA Quantifying Reagent *200X DMSO Solution*

Catalog number: 17597, 17598
Unit size: 1 ml, 10 ml

Component	Storage	Amount (Cat No. 17597)	Amount (Cat No. 17598)
Helixyte™ Green dsDNA Quantifying Reagent *200X DMSO Solution*	Freeze (< -15 °C), Minimize light exposure	1 vial (1 ml)	1 bottle (10 ml)

OVERVIEW

Helixyte™ Green is an excellent nucleic acid sensor that exhibits large fluorescence enhancement upon binding to dsDNA. It has the same spectral properties to those of PicoGreen®, thus a great replacement to PicoGreen® (PicoGreen® is the trademark of Invitrogen). The most commonly used technique for measuring nucleic acid 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, single-stranded nucleic acids and proteins. Helixyte™ Green dsDNA Quantifying Reagent is an excellent alternative for quantifying DNAs with greatly improved sensitivity and selectivity. Helixyte™ Green dsDNA Quantifying Reagent is an ultra-sensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in molecular biological procedures such as cDNA synthesis for library production and DNA fragment purification for subcloning, as well as diagnostic applications, such as quantitating DNA amplification products and primer extension assays. Using the Helixyte™ Green dsDNA Quantifying Reagent, you can selectively detect as little as 25 pg/ml of dsDNA in the presence of ssDNA, RNA, and free nucleotides. 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 Quantifying Reagent has a few orders of magnitude more sensitive than the UV absorbance readings, saving on precious sample. It is specific for dsDNA in the presence of equimolar amounts of RNA.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	515
Emission	525
Excitation	490
Recommended plate	Solid black

PREPARATION OF WORKING SOLUTION

The following example is a protocol for quantifying dsDNA with Helixyte Green™. Before opening, allow the vial of Helixyte Green™ to warm to room temperature.

Note: Exercise caution when working with Helixyte Green™ dsDNA stain, as there is currently no available data regarding its potential mutagenicity or toxicity. Due to its affinity for nucleic acids, this reagent should be handled as a potential mutagen and with appropriate care. Additionally, take special care when handling the DMSO stock solution, as DMSO has been shown to facilitate the entry of organic molecules into tissues.

Preparing the Helixyte Green™ working solution

1. To prepare an aqueous working solution of Helixyte Green™, dilute the concentrated DMSO solution 200-fold in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5-8.0). For example, adding 50 µL of Helixyte Green™ to 10 mL of TE will prepare enough working solution to assay 100 samples in a final volume of 200 µL. 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.

   Note: For best results, this solution should be used within a few hours of its preparation.

SAMPLE EXPERIMENTAL PROTOCOL

Prepare serial dilutions of dsDNA standard (0 to 3 ng/mL)

1. Prepare a 1 mg/mL stock solution of dsDNA (such as calf thymus DNA from Sigma) in ddH_2O

2. To prepare a 10 µg/mL dsDNA solution, add 10 µL of the 1 mg/mL dsDNA stock solution (from Step 2.1) to 990 µL of TE buffer. Next, perform 1:10 and 1:2 serial dilutions to obtain concentrations of 1000, 100, 50, 25, 12.5, 6.25, 3.125, and 0 ng/mL.

3. Add the dsDNA standards and test samples containing DNA into a solid black 96-well microplate, following the instructions provided in Tables 1 and 2.

Table 1. The layout of dsDNA standards and test samples in a solid black 96-well microplate.*

BL	BL	TS	TS	....	....						
DS1	DS1	....	....	....	....						
DS2	DS2	....	....	....	....						
DS3	DS3										
DS4	DS4										
DS5	DS5										
DS6	DS6										
DS7	DS7										

***Note:** DS = dsDNA Standards; BL= Blank Control; TS =Test Samples

Table 2. Reagent composition for each well.*

dsDNA Standard	Blank Control	Test Sample
Serial dilutions* (100 µL)	TE: 100 µL	100 µL

***Note:** Add the serial dilutions of dsDNA standards from 0.1 to 1000 ng/mL into wells from DS1 to DS7 in duplicate.

Run dsDNA assay

1. Add 100 µL of dsDNA assay mixture (from Step 1.1) to each well of the dsDNA standard, blank control, and test samples (see Step 2.3) to make the total dsDNA assay volume of 200 µL/well.

   Note: For a 384-well plate, add 25 µL sample and 25 µL of dsDNA assay mixture per well.

   Note: For cuvette-based assays, add 1mL sample and 1mL of dsDNA assay mixture per cuvette.

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

3. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 490/525 nm, cut off at 515 nm.

4. The fluorescence obtained in the blank wells (containing only TE buffer) is used as a control and will be subtracted from the values obtained in the wells containing dsDNA reactions. The DNA concentrations of the test samples can be determined by referencing the standard curve generated in the DNA Standard Curve step.

EXAMPLE DATA ANALYSIS AND FIGURES

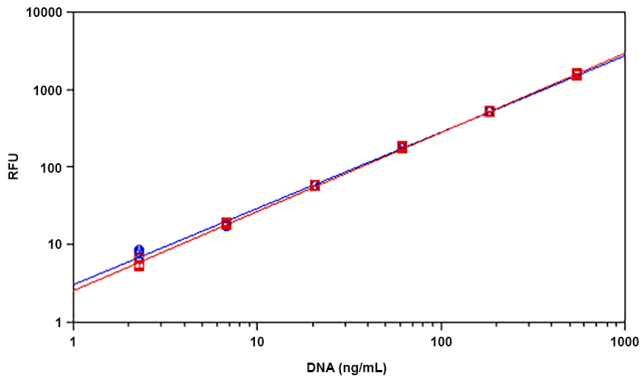

Figure 1. Comparison of calf thymus DNA dose response with Helixyte Green™ (blue circle) and PicoGreen® (red square) in a solid black 96-well microplate using a Gemini fluorescence microplate reader.

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