Helixyte Green™ dsDNA Quantitation Kit

Optimized for fluorescent microplate readers

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 17650 (200 Assays)	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

Helixyte Green™ dsDNA stain is an ultra-sensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in solution. The Helixyte Green™ dsDNA stain has recently been used to quantitate PCR amplification yields in a method for direct cycle sequencing of PCR products. As little as 2.5 ng/ml of dsDNA were detected with minimal effect in the presence of ssDNA, RNA, and free nucleotides, which is comparable with Invitrogen™ Quant-iT™ PicoGreen® dsDNA Reagent. The assay is linear over three orders of magnitude and has little sequence dependence. It is ideal for accurately measuring DNA from many sources, including genomic DNA, viral DNA, mini prep DNA, or PCR.

Kit Components

Components	**Amount**
Component A: Helixyte Green™	100 µL (200X in DMSO)
Component B: Assay buffer	50 mL
Component C: Calf thymus DNA Standard	200 µL (100µg/mL)

Sample Protocol for One 96-well Plate

The following protocol is an example for quantifying dsDNA with Helixyte Green™. Allow all the components to warm to room temperature before opening.

Caution: No data are available addressing the mutagenicity or toxicity of Helixyte Green™dsDNA 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.

1. Preparing Helixyte Green™ working solution

1.1 Prepare Helixyte Green™ working solution by adding 50 µL of Helixyte Green™ (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.

Note1: We recommend preparing this solution in a plastic container rather than glass, as the dye may adsorb to glass surfaces.

Note 2: For best results, this solution should be used within a few hours of its preparation.

2. Prepare serial dilutions of dsDNA standard (0 to 10 µg/mL):

2.1 Add 10 µL of 100µg/mL dsDNA stock solution (Component C) to 190 µL of Assay buffer (Component B) to have 5 µg/mL dsDNA solution, and then perform 1:3 serial dilutions to get 1667, 556, 185, 61.7, 20.6, 6.85, 2.3, and 0 ng/mL.

2.2 Add dsDNA standards and DNA containing test samples into a 96-well solid black microplate as described in Tables 1 and 2.

Table 1. 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 serially dilutions of dsDNA standards from 2.3 to1667 ng/mL into wells from DS1 to DS7 in duplicate.*

3. Run dsDNA assay:

3.1 Add 100 µL of Helixyte Green™ working solution (from Step 1.1) to each well of the dsDNA standard, blank control, and test samples (see Step 2.2) 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 Helixyte Green™ working solution per well.

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

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

3.4 The fluorescence in blank wells (with the TE buffer only) is used as a control, and is subtracted from the values for those wells with the dsDNA reactions. The DNA concentration of the sample are determined from the standard curve generated in *DNA Standard Curve*.

Data Analysis

The fluorescence reading in blank wells (with assay buffer only) is used as a control, and is subtracted from the values of those wells with the dsDNA standards or test samples. A dsDNA standard curve is shown in Figure 1. Calculate the dsDNA concentrations of the samples according to the dsDNA standard curve.

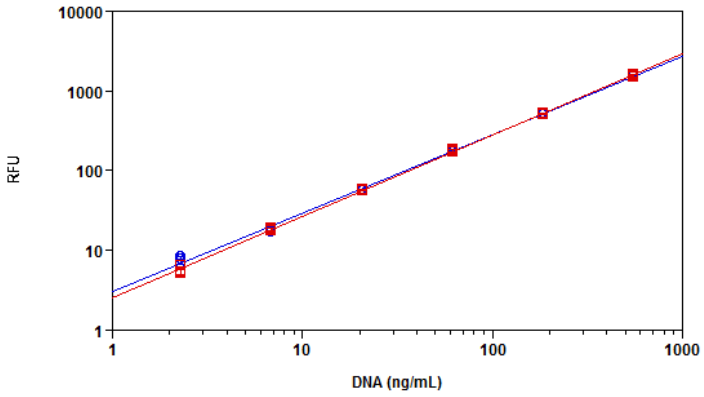

Figure2. Comparison of dsDNA dose response using the Helixyte Green™ (blue circle) with Invitrogen™ Quant-iT™ PicoGreen® dsDNA Reagent (red square). dsNDA standards were stained on a solid black 96-well plate and measured using a Gemini microplate reader (Molecular Devices).

References

1. Chadwick RB, Conrad MP, McGinnis MD. Johnston-Dow L,Spurgeon SL, Kronick MN (1996) Heterozygote and mutation detection by direct automated fluorescent DNA sequencing using a mutant Tag DNA polymerase. Biotechniques 20, 676 (1996).
2. Rye HS, Dabora JM, Quesada MA, Mathies RA, Glazer AN. (1993) Fluorometric assay using dimeric dyes for double- and single-stranded DNA and RNA with picogram sensitivity. Anal Biochem. 208(1):144-50.
3. Ashkin A. (1992) Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime. Biophys J 61, A314.