Helixyte Green™ Nucleic Acid Stain *200X* (Equivalent to PicoGreen®)

Ordering Information	*Storage Conditions*
Product Number: 17597 (1 mL in DMSO, 200X) 17598 (10 mL in DMSO, 200X)	Keep in -20°C. Avoid exposure to light

Biological Applications

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 25 pg/mL of dsDNA (50 pg dsDNA in a 2 mL assay volume) with a standard spectra fluorimeter and 2.5 ng/mL dsDNA with a fluorescent microplate reader were detected with minimal effect in the presence of ssDNA, RNA, and free nucleotides. 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, miniprep DNA, or PCR.

Spectral Properties

Ex/Em = 502/523 nm

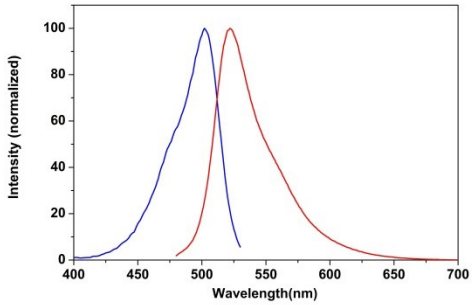

Figure 1. Excitation and emission spectra for the Helixyte Green™ dsDNA stain bound to DNA in PBS (pH 7.4).

Assay Protocol

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

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 the Helixyte Green™ working solution:

1.1 Prepare an aqueous working solution of the Helixyte Green™ by making a 200-fold dilution of the concentrated DMSO solution in TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5–8.0). For example, add 50 µL Helixyte Green™ to 10 mL TE to prepare enough working solution to assay 100 samples in a 200 µL final volume. 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 3 ng/mL):

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

2.2 Add 10 µL of 1 mg/mL dsDNA stock solution (from Step 2.1) to 998 µL TE buffer to have10µg/mL dsDNA solution, and then perform 1:10 and 1:2 serial dilutions to get 1000, 100, 50, 25, 12.5, 6.25, 3.125, and 0 ng/mL.

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

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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 0.1 to1000 ng/mL into wells from DS1 to DS7 in duplicate.*

3. Run dsDNA assay:

3.1 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.
Note1: For a 384-well plate, add 25 µL sample and 25 µL of dsDNA assay mixture per well.
Note2: For curvet based assays, add 1mL sample and 1mL of dsDNA assay mixture per curvet.

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 concentrations of the samples are determined from the standard curve generated in DNA Standard Curve.

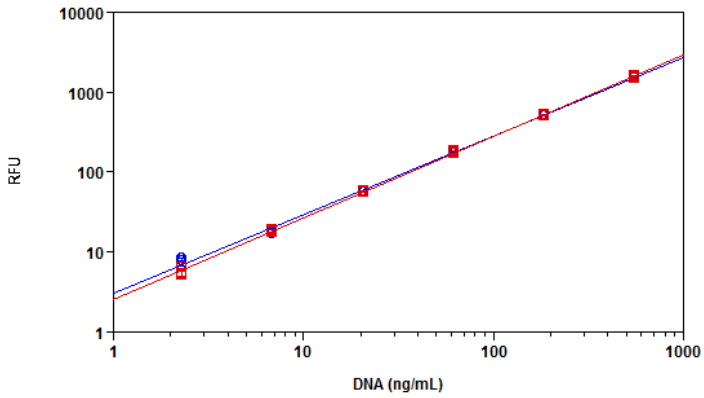

Figure 2. Comparison of calf thymus DNA dose response with Helixyte Green™ (blue circle) and PicoGreen® (red square) in a solid black 96-well microplate using a Gemini fluorescence microplate reader.

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.