Helixyte Green™ dsDNA Quantitation Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 17651 (200 Assays)	Keep in freezer and protect from light	Fluorescence spectrophotometer

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 25 pg/mL of dsDNA (50 pg dsDNA in a 2 mL assay volume) with a standard spectrofluorometer 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™	1 mL (200X in DMSO)
Component B: 20X Assay buffer	25 mL
Component C: Calf thymus DNA Standard	1 mL (100µg/mL)

Sample Protocol

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 1X Assay Buffer

Prepare a 1X Assay buffer by diluting the concentrated buffer 20-fold with sterile, distilled, DNase-free water.

2. Preparing Helixyte Green™ working solution

Prepare Helixyte Green™ working solution by making a 200-fold dilution of the concentrated DMSO solution in 1 X assay buffer. For example, to prepare enough working solution to assay 10 samples in a 2 mL final volume, add 50 µL of Helixyte Green™ (Component A) into 10 mL of Assay Buffer (from Step 1). 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.

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

3.1 For high range standard curve : add 30 µL of 100µg/mL dsDNA stock solution (Component C) to 1.47 mL of 1X Assay buffer (From Step 1) to have 2000 ng/mL dsDNA solution, and then perform 1:2 and 1:10 serial dilutions to get 1000, 100, 10, 1and 0 ng/mL.
3.2 For low range standard curve : add 40 µL of 2µg/mL dsDNA stock solution (From Step 3.1) to 1.56 mL of 1X Assay buffer (From Step 1) to have 50 ng/mL dsDNA solution, and then perform 1:2 and 1:10 serial dilutions to get 25, 2.5, 0.25, 0.025and 0 ng/mL

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

4. Run dsDNA assay:

4.1 Add 1mL of Helixyte Green™ working solution (from Step 2) to each cuvette containing 1 mL of the dsDNA standard, blank control, and test samples to make the total dsDNA assay volume of 2 mL/cuvette.
4.2 Incubate the reaction at room temperature for 5 to 10 minutes, protected from light.
4.3 Monitor the fluorescence increase with a spectroflurometer at Ex/Em = 490/525 nm.
Note: To minimize photobleaching effects, keep the time for fluorescence measurement constant for all samples.
4.4 The fluorescence in blank wells (with the Assay buffer only) is used as a control, and is subtracted from the values for those cuvettes with the dsDNA reactions. The DNA concentration of the samples is determined from the standard curve generated in *DNA Standard Curve*.

Data Analysis

The fluorescence reading in blank cuvette (with assay buffer only) is used as a control, and is subtracted from the values of those cuvettes 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.

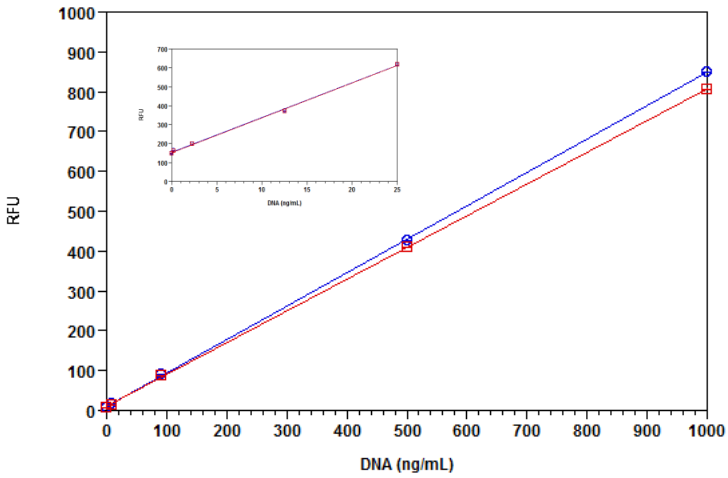

Figure 2. Comparison of dsDNA dose response using the Helixyte Green™ (blue circle) with Invitrogen™ Quant-iT™ PicoGreen® dsDNA Reagent (red square). dsNDA standards were incubated in cuvettes and measured using varian cary eclipse fluorescence spectrophotometer. The inset shows an enlargement of the results obtained with DNA concentrations between zero and 25ng/mL.

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.