



Catalog number: 17660, 17661 Unit size: 100 Tests, 500 Tests

Portelite™ Fluorimetric High Sensitivity DNA Quantitation Kit *Optimized for CytoCite™ and Qubit™ Fluorometers*

Component	Storage	Amount	
		Cat No. 17660	Cat No. 17661
Component A: Helixyte™ Green (200X)	Freeze (<-15 °C), Minimize light exposure	1 vial (0.25 mL-200X in DMSO)	1 vial (1.25 mL-200X in DMSO)
Component B: DNA Assay Buffer	Refrigerate (2-8 °C)	1 bottle (50 mL)	250 mL (3 bottles- 85 mL each)
Component C: DNA Standard #1	Refrigerate (2-8 °C)	1 vial (1 mL, Calf thymus DNA: 0 ng/μL)	1 bottle (5 mL), Calf thymus DNA: 0 ng/μL)
Component D: DNA Standard #2	Refrigerate (2-8 °C)	1 vial (1 mL, Calf thymus DNA: 10 ng/μL)	1 bottle (5 mL), Calf thymus DNA: 10 ng/μL)

OVERVIEW

DNA Quantitation is a very important task in DNA sample preparations for various genomic analyses. This Portelite™ dsDNA Quantitation Kit provides a rapid method to quantify dsDNA with Helixyte™ Green probe using a hand-held fluorometer. It is optimized for Cytocite™ and Qubit™ fluorometers. Portelite™ dsDNA Quantitation assay is linear over five orders of magnitude. The assay is highly selective for double-stranded DNA (dsDNA) over RNA and is designed to be accurate for initial sample concentrations from 25 pg/uL to 100 ng/uL. Helixyte™ Green exhibits large fluorescence enhancement upon binding to dsDNA, and it is a few magnitudes more sensitive than UV absorbance readings.

AT A GLANCE

Protocol summary

- 1. Prepare Helixyte™ Green working solution
- 2. Add 190 µL 1X Helixyte Green[™] working solution into each 0.2 mL PCR tube (Cat#: CCT100)
- 3. Add 10 μL DNA standards or test samples into each tube
- 4. Incubate at room temperature for 2 minutes
- 5. Monitor fluorescence with CytoCite™ fluorometer or Qubit™ fluorometer

Important Bring all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument: CytoCite Fluorometer

Excitation: 480 nm Emission: 520 nm

Instrument specification(s): 0.2 mL, thin-wall PCR tube

Instrument: Qubit Fluorometer

Excitation: 480 nm Emission: 520 nm

Instrument specification(s): 0.2 mL, thin-wall PCR tube

PREPARATION OF WORKING SOLUTION

Helixyte Green™ working solution:

To prepare enough working solution for 10 samples, add 10 μ L of Helixyte GreenTM (Component A) into 2 mL of DNA Assay Buffer (Coponent B).

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

SAMPLE EXPERIMENTAL PROTOCOL

The acceptable sample volume could be a range from 1~20 μ L depending on the estimate concentration of DNA sample. The recommend sample volume is 10 μ L with the DNA concentration in 0.5~10 ng/ μ L range. If other sample volume is being used, please adjust the dilution factor in the concentration calculations.

The following protocol is generated based on 10 μ L sample volume with the DNA concentration in 0.5~10 ng/ μ L range.

 Add 190 µL 1X Helixyte Green™ working solution into each Cytocite™ sample tube (#CCT100) or equivalent 0.2 mL PCR tube

Note Use thin-wall, polypropylene, clear 0.2 mL PCR tubes such as #CCT100.

- 2. Add DNA standards or test samples 10 μL into each tube, and then mix by vortexing 2~3 seconds.
- 3. Allow all tubes to incubate at room temperature for 2 minutes.
- Insert the samples into CytoCite™ or Quibit™ and monitor the fluorescence with green fluorescence channel. Follow the procedure appropriate for CytoCite™ Fluorometer.

Note See the link below for detailed instructions: https://devices.aatbio.com/documentation/user-manual-for-cytocite-fluorometer

PREPARATION OF STANDARD Calibration Curve

For Portelite™ assays, you have the choice to make a calibration curve with the DNA standards. Here is a brief protocol to generate a customized DNA standard curve:

- 1. Perform dilution with DNA Assay Buffer to get 10, 8, 6, 4, 2, 1, 0.5, 0 $ng/\mu L$ DNA standard dilutions.
- 2. Add 190 μL of Helixyte Green $^{\intercal\!\!M}$ working solution into a 0.2 mL PCR tube.
- 3. Add 10 μL standards or 10 μL samples into each tube.
- 4. Incubate the reaction at room temperature for 2 minutes.
- 5. Insert the samples into CytoCite™ and monitor the fluorescence with green fluorescence channel.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Concentration samples. We recommend using the Online Linear Regression Calculator which can be found at:

 ${\color{blue} \underline{https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator} \\$

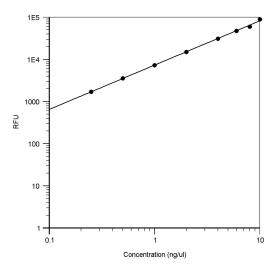


Figure 1. DNA standard curve generated using Portelite™ Fluorimetric DNA High Sensitivity Quantitation Kit. Fluorescence intensity was quantified using FITC channel, regression model was calculated using log-log best-fit. Detection limit was $10 \text{ pg}/\mu\text{L}$.

DISCLAIMER

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