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

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

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 (2-4 °C)	1 bottle (50 mL)	250 mL (3 bottles- 85 mL each)
Component C: DNA Standard #1	Refrigerate (2-4 °C)	1 vial (1 mL)	1 bottle (5 mL)
Component D: DNA Standard #2	Refrigerate (2-4 °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 µL of Helixyte Green™ (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 µL depending on the estimate concentration of DNA sample. The recommend sample volume is 10 µL with the DNA concentration in 0.5~10 ng/ µL 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 µL sample volume with the DNA concentration in 0.5~10 ng/µL range.

1. 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.

4. 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/µL DNA standard dilutions.

2. Add 190 µL of Helixyte Green™ 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.

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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 base-line 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:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

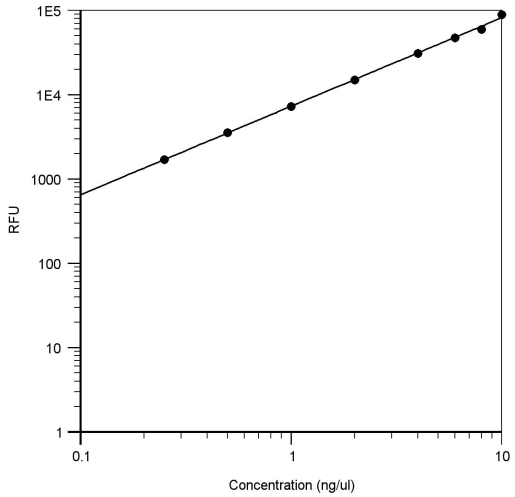

Figure 1. DNA standard curve generated using Portelite™ Fluorimetric DNA High Sensitivity Quantitation Kit. Fluorescence intensity was quantified using FITC channel, regression model was calculated using log-log best-fit. Detection limit was 10 pg/µL.

DISCLAIMER