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)	Amount (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	Freeze (< -15 °C)	1 bottle (50 mL)	250 mL (3 bottles - 85 mL each)
Component C: DNA Standard #1	Freeze (< -15 °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	Freeze (< -15 °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 1.25 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 Note

Bring kit components to room temperature before starting the experiment.

PREPARATION OF WORKING SOLUTION

Helixyte Green™ working solution

1. To prepare enough working solution for 10 samples, add 10 µL of Helixyte Green™ (Component A) to 2 mL of DNA Assay Buffer (Component 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

Important

The acceptable sample volume could range from 1~20 µL depending on the estimated concentration of the DNA sample. The recommended sample volume is 10 µL with the DNA concentration in 0.5~10 ng/ µL range. If another sample volume is being used, please adjust the dilution factor in the concentration calculations.

1. Add 190 µL of 1X Helixyte Green™ working solution to each Cytocite™ sample tube (#CCT100) or an 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 a 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

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 a green fluorescence channel.

EXAMPLE DATA ANALYSIS AND FIGURES

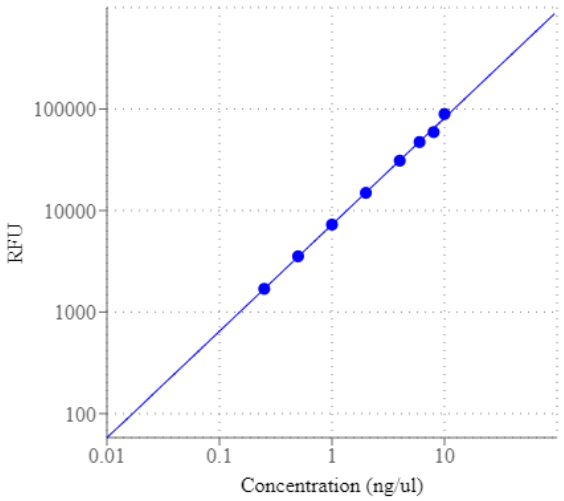

Figure 1. DNA standard curve generated using Portelite™ Fluorimetric DNA High Sensitivity Quantitation Kit. Fluorescence intensity was quantified using FITC channel, regression model was calculated using log-log best-fit. Detection limit was 10 pg/µL.

DISCLAIMER

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