Portelite™ Fluorimetric DNA Quantitation Kit with Broad Dynamic Range *Optimized for Cytocite™ and Qubit™ Fluorometers*

Catalog number: 17665
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 17665)
Component A: Helixyte™ Green BR	Freeze (< -15 °C), Minimize light exposure	1 vial (0.25 mL, 200X in DMSO)
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (50 mL)
Component C: DNA Standard BR #1	Refrigerated (2-8 °C)	1 vial (1 mL, Calf thymus DNA: 0 ng/uL)
Component D: DNA Standard BR #2	Refrigerated (2-8 °C)	1 vial (1 mL, Calf thymus DNA: 100 ng/uL)

OVERVIEW

DNA Quantitation is a very important task in DNA sample preparations for various analyses. Portelite™ Fluorimetric DNA Quantitation Kit provides a rapid method to quantify dsDNA with Helixyte™ Green BR using a hand-held fluorometer. It is optimized for Cytocite™ and Qubit™ fluorometers. Portelite™ Fluorimetric DNA Quantitation assay is linear over three orders of magnitude. The assay is highly selective for double-stranded DNA (dsDNA) over RNA and is optimized for measuring dsDNA concentrations from 10 pg/µL to 100 ng/µL. Helixyte™ Green BR 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 BR working solution
2. Add 190 uL 1X Helixyte™ Green BR working solution into each 0.2 mL PCR tube
3. Add 10 uL DNA standards or test samples into each tube
4. Incubate at room temperature for 2 minutes
5. Monitor fluorescence with CytoCite™ fluorometer or Qubit™

Important Note

Bring all the components at room temperature before opening.

PREPARATION OF WORKING SOLUTION

Helixyte™ Green BR working solution

Make a 200-fold dilution of Portelite™ dsDNA reagent (Component A) in DNA Assay Buffer (Component B). For example, to prepare enough working solution for 8 samples, add 5 uL of Helixyte™ Green BR (Component A) into 1 mL of DNA Assay Buffer (Component B). Protect the working solution from light by covering it with foil or placing it in the dark.

Note: Protect the working solution from light by covering it with foil or placing it in the dark. 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 uL depending on the estimate concentration of DNA sample. The recommend sample volume is 10 uL with the DNA concentration in 0.2~100 ng/uL 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 uL sample volume with the DNA concentration in 0.2~100 ng/uL range.

1. Add 190 uL 1X Helixyte™ Green BR 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 uL into each tube, and then mix by vortexing 2~3 seconds.
3. Incubate all tubes 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. See the link below for detailed instructions: https://devices.aatbio.com/documentation/user-manual-for-cytocite-fluorometer

PREPARATION OF STANDARD CALIBRATION CURVE

1. Perform 1/3 serial dilution with 100 ng/uL with DNA Standard BR #2 (Component D) in DNA Assay Buffer (Component B) to get 30, 10, 3, 1, 0.3, 0.1 and 0 ng/uL DNA standard dilutions.
2. Add 190 uL of Helixyte™ Green BR working solution into each tube.
3. Add 10 uL standards or 10 uL samples into a 0.2 mL PCR 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 DNA 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

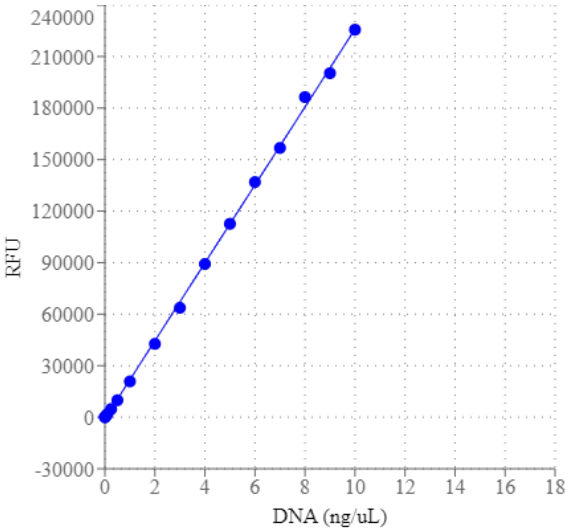

Figure 1. DNA standard curve generated using Portelite™ Fluorimetric DNA Quantitation Kit with Broad Dynamic Range. Fluorescence intensity was quantified using green fluorescence channel, regression model was calculated using linear fit.

DISCLAIMER

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