Portelite™ Fluorimetric ssDNA Quantitation Kit *Optimized for Cytocite™ and Qubit™ Fluorometers*

Catalog number: 17625
Unit size: 200 Tests

Component	Storage	Amount
Component A: Helixyte™ Green ssDNA	Freeze (< -15 °C), Minimize light exposure	1 vial (0.25 mL-200X DMSO)
Component B: Assay Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (100 mL)
Component C: ssDNA Standard #1	Refrigerated (2-8 °C)	1 vial (1 mL: 0 ng/µL)
Component D: ssDNA Standard #2	Refrigerated (2-8 °C)	1 vial (1 mL, 10 ng/µL)

OVERVIEW

Portelite™ Fluorimetric ssDNA Quantitation Kit is designed to rapidly measure single-stranded DNA. The kit has all the essential reagents, including Helixyte™ Green ssDNA reagent, dilution buffer, and prediluted DNA standards. Helixyte™ Green ssDNA reagent is a sensitive fluorescent nucleic acid probe for quantifying oligonucleotides and single-stranded DNA (ssDNA) in solution. Simply dilute the reagent using the buffer provided, add your sample (any volume from 1–20 µL is acceptable), then read the concentration using CytoCite, Qubit® or other hand-held or desk top fluorometers (Qubit® is the trademark of ThermoFisher). The assay is accurate for initial sample concentrations from 50 pg/µL to 200 ng/µL, providing an assay range of 1–200 ng. The assay detects long oligonucleotides or ssDNA. Nucleotides and short oligo nucleotides of six bases or less do not interfere in the quantitation assay. The detection limit is not significantly interfered by the common contaminants in nucleic acid preparations, including salts, urea, ethanol, chloroform, detergents, proteins, nucleotides and short oligonucleotides of six bases. However, double-stranded DNA (dsDNA) and RNA do interfere with the assay as Helixyte™ Green ssDNA reagent binds to dsDNA and RNA to generate additional fluorescence signal. Portelite™ Fluorimetric ssDNA Quantitation Kit is optimized for CytoCite™ and Qubit® fluorometers.

AT A GLANCE

Protocol summary

1. Prepare the Helixyte™ Green ssDNA working solution
2. Add 190 µL of 1X Helixyte™ Green ssDNA working solution into each 0.2 mL PCR tube
3. Add 10 µL of ssDNA Standards or test samples into each tube
4. Incubate at room temperature for 2 minutes
5. Monitor the fluorescence intensity with CytoCite™ fluorometer or Qubit™ fluorometer

Important
All kit components must be brought to room temperature before starting the experiment.

KEY PARAMETERS

Qubit Fluorometer

Excitation	480 nm
Emission	530 nm
Instrument specification(s)	0.2 mL PCR vial

CytoCite Fluorometer

Excitation	480 nm
Emission	530 nm
Instrument specification(s)	0.2 mL PCR vial

PREPARATION OF WORKING SOLUTION

Helixyte™ Green ssDNA working solution

Make a 200-fold dilution of Helixyte™ Green ssDNA reagent (Component A) with Assay Buffer (Component B). For example, to prepare enough working solution for 5 samples, add 5 µL of Helixyte™ Green ssDNA (Component A) into 1 mL of Assay Buffer (Component B).

Note Protect the working solution from light by covering it with foil or placing it in the dark. It's recommended to prepare the solution in a plastic container rather than a glass container, as the dye may adsorb to the glass surface. For best results, this solution should be used within a few hours after the dilution.

SAMPLE EXPERIMENTAL PROTOCOL

The acceptable range for the sample volume could be 1~20 µL depending on the estimated concentration of the Nucleic Acid sample.
The following protocol is generated based on a sample volume of 10 µL with the DNA concentration in the range of 20~1000 ng /mL.

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

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

2. Add 10 µL of ssDNA Standards or test samples into each tube, and then mix by vortexing for 2~3 seconds.

3. Incubate all tubes at room temperature for 2 minutes.

4. Insert the samples into CytoCite™ or Qubit™ and monitor the fluorescence intensity with the green fluorescence channel. Follow the appropriate procedures for the 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 a 1:2 serial dilution: Add 10 ng/µL ssDNA Standard #2 (Component D) into Assay Buffer (Component B) to get 10, 5, 2.5, 1.25, 0.62, 0.31, 0.15 ng/µL DNA standard dilutions.

2. Add 190 µL of the Helixyte™ Green ssDNA working solution into each tube.

3. Add 10 µL of standards into a 0.2 mL PCR tube and then mix by vortexing for 2~3 seconds.

4. Incubate the reaction at room temperature for 2 minutes.

5. Insert the samples into CytoCite™ and monitor the fluorescence intensity with the green fluorescence channel.

EXAMPLE DATA ANALYSIS AND FIGURES

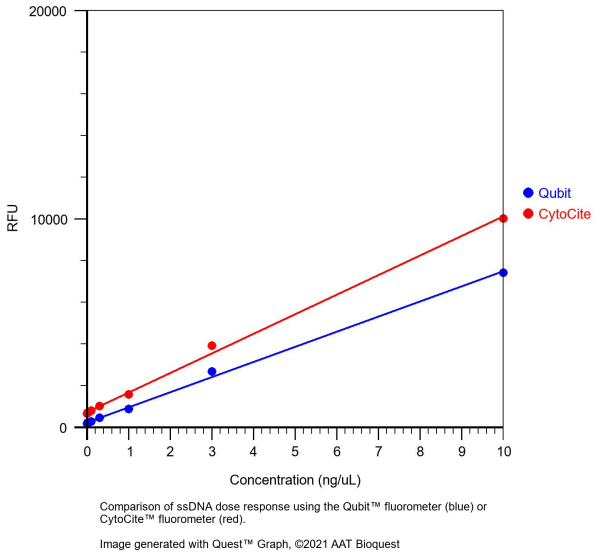

Figure 1. Comparison of ssDNA dose response using the Qubit™ fluorometer (blue) or CytoCite™ fluorometer (red).

DISCLAIMER

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