Portelite™ Fluorimetric Total Nucleic Acid Quantitation Kit *Optimized for Cytocite™ and Qubit™ Fluorometers*

Catalog number: 17631
Unit size: 200 Tests

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

OVERVIEW

Portelite™ Fluorimetric Total Nucleic Acid Quantitation Kit is designed to rapidly measure the total amounts of nucleic acids, including double-stranded DNA (dsDNA), single-stranded DNA (ssDNA) and RNA in a sample. The kit has all the essential reagents, including Helixyte™ Green ssDNA reagent, dilution buffer, and prediluted DNA standards. Helixyte™ Green All reagent is a sensitive fluorescent nucleic acid probe for measuring the total amounts of nucleic acids in a sample that may contain double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), RNA and long oligonucleotides. Helixyte™ Green All reagent indiscriminately binds to dsDNA, ssDNA and RNA. Portelite™ Fluorimetric Total Nucleic Acid Quantitation Kit is optimized for measuring the total amounts of nucleic acids with CytoCite™ or Qubit® fluorometers.

AT A GLANCE

Protocol Summary

1. Prepare a Helixyte™ Green All working solution
2. Add 190 µL of 1X Helixyte™ Green All working solution into each 0.2 mL PCR tube
3. Add 10 µL of Nucleic Acid 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 tube

CytoCite Fluorometer

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

PREPARATION OF WORKING SOLUTION

Helixyte™ Green All working solution

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

1. Add 190 µL of 1X Helixyte™ Green All 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 Nucleic Acid 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 CytoCite™ Fluorometer. 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 Nucleic Acid Standards. Here is a brief protocol to generate a customized DNA standard curve.

1. Perform a 1:2 serial dilution: Add 10 ng/µL Nucleic Acid Standard #2 (Component D) into the 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 All 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

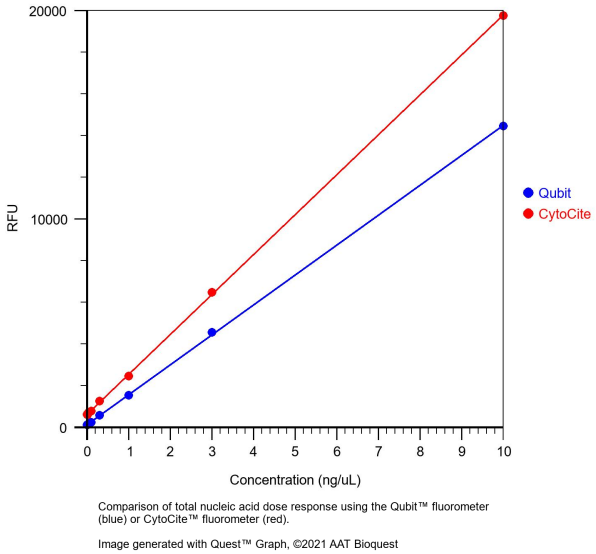

Figure 1. Comparison of total nucleic acid dose response using the Qubit™ fluorometer (blue) or CytoCite™ fluorometer (red).

DISCLAIMER

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