Portelite™ Rapid Fluorimetric Biotin Quantitation Kit *Optimized to Use with CytoCite™ and Qubit™ Fluorometers*

Catalog number: 5539
Unit size: 50 tests

Component	Storage	Amount (Cat No. 5539)
Component A: Biotinylite™ Green (10X)	Freeze (< -15 °C), Minimize light exposure	2 Vials (0.5 mL/Vial)
Component B: Biotin Standard (300 µM)	Freeze (< -15 °C)	2 Vials (100 µL/Vial)
Component C: Assay Buffer	Freeze (< -15 °C), Minimize light exposure	1 Bottle (25 mL)

OVERVIEW

Portelite™ Rapid Fluorimetric Biotin Quantitation Kit is specifically optimized to quantify biotin and biotin conjugates with a portal fluorometer such as CytoCite™ and Qubit™ Fluorometer. The kit uses Biotinylite™ Green, a fluorogenic biotin sensor. Biotinylite™ Green is almost non-fluorescent and give strong green fluorescence upon interaction with a biotin or biotin conjugate. The concentration of biotin is proportional to the fluorescence intensity of Biotinylite™ Green. The amount of biotin is determined by comparing a sample's fluorescence to the predetermined biotin standard curve. This fluorescence-based assay is much more sensitive than the commonly used colorimetric HABA assay. Biotin is a relatively small molecule that is routinely conjugated to antibodies and proteins with minimal interference of their biological activity. The avidin/streptavidin-biotin interaction is the strongest known binding pair between a protein and its ligand. The biotin-avidin interaction has been extensively explored for a variety of biological applications.

KEY PARAMETERS

Qubit Fluorometer

Emission	530 nm
Excitation	490 nm
Instrument specification(s)	Green Fluorescent Channel

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/5539

Biotin Standard
Add 10 µL of the 300 µM Biotin Standard stock solution to 90 µL of Assay Buffer (Component C) to create a 30 µM Biotin standard (STD7). Refer to Table 1 for detailed serial dilution instructions.

PREPARATION OF WORKING SOLUTION

Biotinylite™ Green Working Solution

1. To prepare the Biotinylite™ Green working solution, add 100 µL of Biotinylite™ Green (10X) (Component A) into 900 µL of Assay Buffer (Component C), and mix thoroughly.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Preparation of Biotin Standards for thin-wall PCR tubes (Working Range: 0.75-30 µM in 20 µL).

Vial	Assay Buffer (µL)	Biotin Standard (30 µM)	Final Biotin Conc. in 20 µL (µM)
STD7	0	20	30
STD6	4	16	24
STD5	12	8	12
STD4	16	4	6
STD3	18	2	3
STD2	19	1	1.5
STD1	19.5	0.5	0.75
BL	20	0	0

Table 2. Preparation of Biotin Sample for thin-wall PCR tubes (Working Range: 0.75-30 µM in 20 µL).

Vial	Assay Buffer (µL)	Test Sample	Final Biotin Conc. in 20 µL (µM)
TS	(20-V) µL	V µL	Dilute to 1 ~ 30 µM

Note: Please estimate the concentration of biotin in your sample. Dilute it to achieve a concentration within the range of 1 to 30 µM. Adjust the volume to 20 µL using Assay Buffer (Component C).

Example: For a 1 mg/mL IgG-Biotin sample with a Biotin number of approximately 6.0, the Biotin concentration is around 40 µM. Therefore, you should use 5.0 µL of the sample and add 15.0 µL of Assay buffer (Component C) to achieve a final Biotin concentration of approximately 10 µM.

Run Biotin Assay

1. Dispense 180 µL of Biotinylite™ Green working solution into each tube.

2. Incubate the reaction at room temperature for 30 to 60 minutes.

3. Insert the samples into the CytoCite™ or Qubit® fluorimeters and monitor the fluorescence using the green fluorescent channel. For operational instructions for the CytoCite™, please refer to the following link:

   https://devices.aatbio.com/documentation/user-manual-for-cytocite-fluorometer

Sample Protocol for Qubit® Fluorimeter

1. On the Qubit® Home screen, select "Protein" and then choose "Read standards."

2. Insert each tube containing the standards into the sample chamber.

3. Close the lid and press the "Read Standards" button.

4. The instrument presents the measurements for each standard and sample.

5. Plot the standard curve, and use it to calculate the concentration of your samples.

EXAMPLE DATA ANALYSIS AND FIGURES

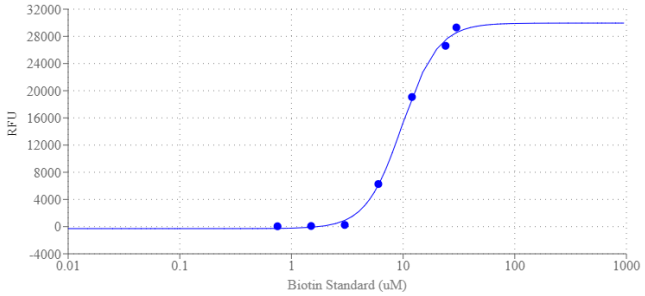

Figure 1. The Biotin Standard Curve was determined using the Portelite™ Rapid Fluorimetric Biotin Quantitation Kit, with measurements taken on a Qubit™ 4 Fluorometer using the blue (470 nm) channel.

DISCLAIMER

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