Amplite® Fluorimetric Biotin Quantitation Kit

Catalog number: 5538
Unit size: 100 tests

Component	Storage	Amount (Cat No. 5538)
Component A: Biotinylite™ Green (10X)	Freeze (< -15 °C), Minimize light exposure	1 Vial (0.5 mL)
Component B: Biotin Standard	Freeze (< -15 °C)	1 Vial(1 µL/Vial, 300 µM)
Component C: Assay Buffer	Freeze (< -15 °C), Minimize light exposure	1 Bottle (25 mL)

OVERVIEW

Amplite® Fluorimetric Biotin Quantitation 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

Fluorescence microplate reader

Cutoff	515 nm
Emission	530 nm
Excitation	490 nm
Recommended plate	Solid black

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/5538

Biotin Standard
To prepare a 6 µM Biotin Standard (STD7), add 10 µL of 300 µM Biotin Standard stock solution to 490 µL of Assay Buffer (Component C). Then, create 1:2 serial dilutions by taking 200 µL of this Biotin Standard solution and diluting it with equal volumes of Assay Buffer (Component C) to obtain serially diluted standards (STD7-STD1).

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) to 900 µL of Assay Buffer (Component C) and mix thoroughly.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of biotin standards and test samples in a solid black 96-well microplate.

STD = Biotin Standard (STD1-STD7, 0.094 ~ 6 µM), **BL** = Blank Control, **TS** = Test Samples

STD7	STD7	TS1	TS1
STD6	STD6	TS2	TS2
STD5	STD5	...	...
STD4	STD4	...	...
STD3	STD3	...	...
STD2	STD2	...	...
STD1	STD1	...	...
BL	BL	...	...

Table 2. Reagent composition for each well.

Well	Volume	Reagents
STD7~STD1	50 µL	Biotin Serial Dilution (0.094 - 6 µM)
BL	50 µL	Assay Buffer Control
TS	50 µL	Test Sample

1. Prepare the Biotin standard (STD1~STD7), blank controls (BL), and test samples (TS) as outlined in Tables 1 and 2, using 25 µL of reagent per well for a 384-well plate instead of 50 µL.

2. Add 50 µL of Biotinylite™ Green working solution to each well containing Biotin standards (STD1~STD7), blank controls (BL), and test samples (TS), ensuring the total volume in each well reaches 100 µL. For a 384-well plate, use 25 µL of the working solution per well.

3. Incubate the reaction at room temperature for 10 to 30 minutes, protected from light.

4. Monitor the fluorescence increase at Ex/Em = 490/530 nm (Cutoff = 515 nm) with a fluorescence microplate reader.

EXAMPLE DATA ANALYSIS AND FIGURES

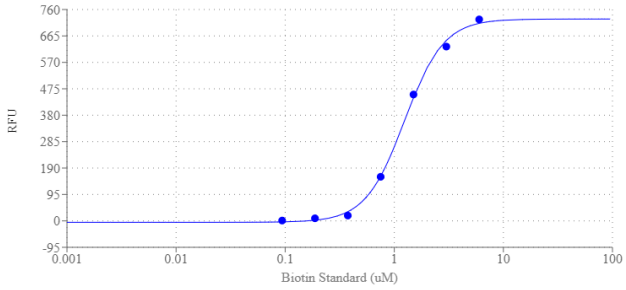

Figure 1. The Biotin Standard Curve was determined using a black bottom 96-well plate and the Amplite® Fluorimetric Biotin Quantitation Kit, with measurements taken on a fluorescence microplate reader at an Ex/Em = 490/530 nm and Cutoff = 515 nm.

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