Amplite™ Fluorimetric Formaldehyde Quantitation Kit *Green Fluorescence*

Catalog number: 10057
Unit size: 200 Tests

Component	Storage	Amount
Component A: AldeLight™ Green	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (30 mL)
Component C: 37.2% Formaldehyde Standard (12.3 M)	Freeze (<-15 °C)	1 vial (100 µL)
Component D: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

Formaldehyde is a naturally occurring substance. Natural processes in the upper atmosphere may contribute up to 90 percent of the total formaldehyde in the environment. Formaldehyde, as well as its oligomers and hydrates are rarely encountered in living organisms. Methanogenesis proceeds via the equivalent of formaldehyde, but this one-carbon species is masked as a methylene group in methanopterin. Formaldehyde is the primary cause of methanol's toxicity, since methanol is metabolized into toxic formaldehyde by alcohol dehydrogenase. Our Amplite™ Fluorimetric Formaldehyde Quantitation Kit are used for quantifying formaldehyde. The kit uses a proprietary fluorogenic dye that generates a strongly fluorescent product upon reacting with formaldehyde. This fluorimetric kit provides a sensitive mix-and-read method to detect formaldehyde. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read with a fluorescence microplate reader.

AT A GLANCE

Protocol summary

1. Prepare Formaldehyde standards and/or test samples (50 µL)
2. Add AldeLight™ Green working solution (50 µL)
3. Incubate at RT for 20 to 60 minutes
4. Monitor fluorescence increase at Ex/Em = 410/525 nm (Cutoff = 495 nm)

Important Thaw all the kit components to room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	410 nm
Emission:	525 nm
Cutoff:	495 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. AldeLight™ Green stock solution (500X):
Add 20 µL of DMSO (Component D) into the vial of AldeLight™ Green (Component A) to make 500X AldeLight™ Green stock solution.

2. Formaldehyde stadard solution (123 mM):
Add 5 µL of 37.2% of Formaldehyde Standard (Component C) into 0.5 mL of Assay Buffer (Component B) to make 123 mM Formaldehyde standard solution.

PREPARATION OF STANDARD SOLUTION

Formaldehyde standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/10057

Add 12.2 µL of 123 mM Formaldehyde standard solution into 0.5 mL of Assay Buffer (Component B) to make 3 mM Formaldehyde standard solution. Take 3 mM Formaldehyde standard solution and perform 1:10 in Assay Buffer (Component B) to make 300 µM Formaldehyde standard (FS7). Take 300 µM Formaldehyde standard (FS7) and perform 1:3 serial dilutions to get serially diluted Formaldehyde standards (FS6-FS1) with Assay Buffer (Component B).

PREPARATION OF WORKING SOLUTION

Add 10 µL of 500X AldeLight™ Green stock solution into 5 mL of Assay Buffer (Component B) and mix well to make AldeLight™ Green working solution.

Note 5 mL of AldeLight™ Green working solution is enough for 1 plate. AldeLight™ Green working solution is not stable, and best used within 2 hours.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Formaldehyde standards and test samples in a solid back 96-well microplate. FS= Formaldehyde Standards (FS1 - FS7, 0.41 to 300 µM), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
FS1	FS1	...	...
FS2	FS2	...	...
FS3	FS3		
FS4	FS4		
FS5	FS5		
FS6	FS6		
FS7	FS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
FS1 - FS7	50 µL	Serial Dilutions (0.41 to 300 µM)
BL	50 µL	Assay Buffer
TS	50 µL	test sample

1. Prepare Formaldehyde standards (FS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of AldeLight™ Green working solution to each well of Formaldehyde standard, blank control, and test samples to make the total Formaldehyde assay volume of 100 µL/well. For a 384-well plate, add 25 µL of

AldeLight™ Green working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 20 to 60 minutes, protected from light.

4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 410/525 nm (Cutoff = 495nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Response 1) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Concentration 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

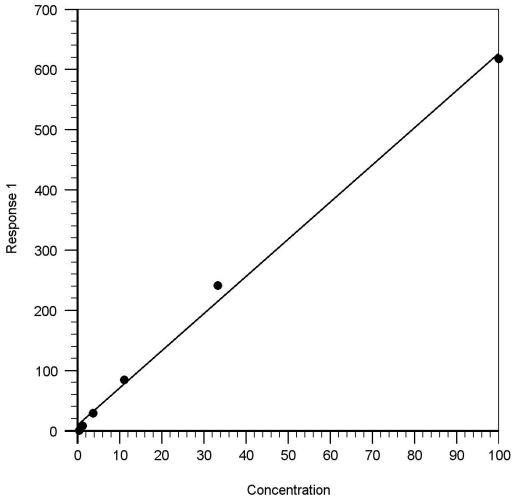

Figure 1.

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