Amplite™ Fluorimetric Formaldehyde Quantitation Kit
Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 10057 (200 assays)	Keep at -20 ^{o}C, avoid light	Fluorescence microplate readers

Introduction

Formaldehyde is one of the well-identified volatile chemical contaminants responsible for indoor pollution and "building sick" syndrome disease and was recently classified as carcinogenic. The main sources for the HCHO pollution in air include painting, coating material and cigarette smoking. Rapid and accurate measurement of formaldehyde is an important task for biological research, food industry, chemical research and environmental pollution surveillance. There are a few reagents or assay kits available for quantifying formaldehyde. Most of the existing aldehyde test methods are based on separations either by the tedious and expensive HPLC-MS or GC-MS. Our Amplite™ Fluorimetric Formaldehyde Quantitation kit uses a proprietary fluorogenic dye that generates a green fluorescent product upon reacting with formaldehyde. The kit provides a sensitive, one-step fluorometric method to detect as little as 1 uM of formaldehyde in a 100 µL assay volume (Figure 1). 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 by a fluorescence microplate reader at Ex/Em = 410/525 nm.

Kit Key Features

Broad Application:	Quantify formaldehyde in a variety of applications such as enzyme reactions.
Sensitive:	Detect as low as 1uM of formaldehyde in a 100 µL assay volume.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: AldeLight™ Green	1 vial
Component B: Assay Buffer	1 bottle (30 mL)
Component C: 37.2% Formaldehyde Standard (12.3 M)	1 vial (100 µL)
Component D: DMSO	1 vial (100 µL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare enzyme reaction solution (50 µL) → Add AldeLight™ Green reaction mixture (50 µL)

→ Incubate at RT for 20 to 60 minutes → Read Fluorescence at Ex/Em = 410/525 nm

Note: Thaw all the kit components to room temperature before starting your experiment.

1. Prepare 500X AldeLight™ Green stock solution:

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

Note: The unused AldeLight™ Green solution should be aliquoted, and stored at -20°C (avoid light).

2. Prepare AldeLight™ Green reaction mixture:

>   Add 10 µL of 500X AldeLight™ Green (from Step 1) into 5 mL of Assay Buffer (from Component B), mix well.
>   *Note: 5 mL of AldeLight™ Green reaction mixture is enough for 1 plate. The reaction mixture is not stable, and best used within 2 hours.*

3. Prepare serial formaldehyde standard (0 to 300 µM) solutions:

>   3.1   Add 5 µL of 37.2% Formaldehyde Standard (Component D) into 0.5 mL of Assay Buffer (from Component B) to make 123 mM stock solution.
>
>   3.2   Add 12.2 µL of 123 mM Formaldehyde Standard Solution (from Step 3.1) into 0.5 mL of Assay Buffer (from Component B) to make 3 mM stock solution.
>
>   3.3   Take 3 mM Formaldehyde Standard Solution (from Step 3.2) to perform 1:10, and 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1, 0.3, 0.1, and 0 µM standard formaldehyde solutions.
>
>   3.4   Add formaldehyde standards and formaldehyde-containing test samples into a 96-well black solid microplate as described in Tables 1 and 2

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

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

Note: FS= Formaldehyde Standards, BL=Blank Control, TS=Test Samples.

Table 2. Reagent composition for each well

Formaldehyde Standard	Blank Control	Test Sample
Serial dilutions* (50 µL)	Assay buffer: 50 µL	50 µL

Note: Add the serially diluted formaldehyde standards from 0.1 µM to100 µM into wells from FS1 to FS7 in duplicate.

4. Run formaldehyde assay:

>   4.1   Add 50 µL of AldeLight™ Green reaction mixtures (from Step 2) to each well of the formaldehyde standard, blank control, and test samples (see Step 3.4) to make the total formaldehyde assay volume of 100 µL/well.
>   *Note: For 384-well plate add 25 µL of test sample and 25 µL of AldeLight™ Green reaction mixtures into each well.*
>
>   4.2   Incubate the reaction mixture at room temperature for 20 to 60 minutes (protected from light).
>
>   4.3   Monitor the fluorescence increase at Ex/Em = 410/525 nm using a fluorescence plate reader.

Data Analysis

The fluorescence in blank wells (with 0 µM Formaldehyde Standard and AldeLight™ Green reaction mixture only) is used as a control, and subtracted from the values for those wells with the formaldehyde reactions. A formaldehyde standard curve is shown in Figure 1.
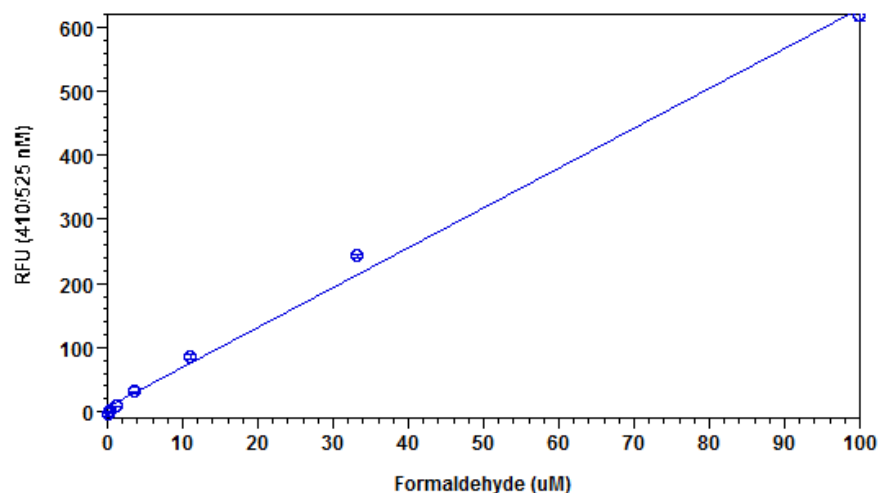
Figure 1. Formaldehyde dose response was measured in a 96-well black plate with Amplite™ Fluorimetric Formaldehyde Quantitation Kit using a Gemini fluorescence microplate reader (Molecular Devices). As low as 1 µM of formaldehyde can be detected with 30 minutes incubation time (n=3). *Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.*

References

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