

## Amplite™ Fluorimetric Aldehyde Quantitation Kit

### \*Blue Fluorescence\*

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 10052 (200 assays)	Keep at -20 °C Avoid exposure to light	Fluorescence microplate readers

### Introduction

The formation, reactivity and toxicity of aldehydes originating from the peroxidation of lipids of cellular membranes have received great attention in recent years. Rapid and accurate measurement of aldehydes is an important task for biological research, chemical research, food industry and environmental pollution surveillance. There are a few reagents or assay kits available for quantifying the number of aldehydes. Most of the existing aldehyde test methods are based on separations either by the tedious and expensive HPLC-MS or GC-MS.

Both our Amplite™ Colorimetric Aldehyde Quantitation Kit (10051) and Amplite™ Fluorimetric Aldehyde Quantitation kit (10052) are used for quantifying aldehydes at higher pH. Kit 10052 uses a proprietary fluorogenic dye that generates a strongly fluorescent product upon reacting with an aldehyde. Kit 10052 is much more sensitive than Kit 10051. This fluorimetric kit provides a sensitive mix-and-read method to detect as little as 0.3 nanomole of aldehyde in a 100 µL assay volume (3 µM). 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 read by a fluorescence microplate reader at Ex/Em = 365/435 nm.

### Kit Key Features

<b>Broad Application:</b>	Used for quantifying aldehydes in a variety of applications, such as enzyme reactions.
<b>Sensitive:</b>	Detect as low as 0.3 nanomole of aldehyde in a 100 µL assay volume.
<b>Continuous:</b>	Easily adapted to automation without a separation step.
<b>Convenient:</b>	Formulated to have minimal hands-on time.
<b>Non-Radioactive:</b>	No special requirements for waste treatment.

### Kit Components

Components	Amount
Component A: AldeLight™ Blue	1 vial
Component B: Assay Buffer	1 bottle (30 mL)
Component C: Reaction Buffer	1 vial (6 mL)
Component D: Aldehyde Standard	1 vial
Component E: DMSO	1 vial (100 µL)

### Assay Protocol for One 96-Well Plate

#### Brief Summary

**Prepare enzyme reaction solution (50 µL) → Add AldeLight™ Blue reaction mixture (50 µL)**  
**→ Incubate at RT for 15 to 30 minutes → Add 25 µL of Reaction Buffer**  
**→ Monitor fluorescence increase at Ex/Em = 365/435 nm**

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

**1. Prepare 250X AldeLight™ Blue stock solution:**

Add 40 µL of DMSO (Component E) into the vial of AldeLight™ Blue (Component A) to make 250X AldeLight™ Blue stock solution.

*Note: The unused AldeLight™ Blue stock solution should be divided into single use aliquots, and stored at -20°C.*

**2. Prepare AldeLight™ Blue reaction mixture:**

Add 20 µL of 250X AldeLight™ Blue stock solution (from Step 1) into 5 mL of Assay Buffer (Component B), and mix them well.

*Note: 5 mL of AldeLight™ Blue reaction mixture is enough for one plate. The reaction mixture is not stable, and best used within 2 hours.*

**3. Prepare serial dilutions of aldehyde standard (0 to 1 mM):**

3.1 Add 1 mL of Assay Buffer (Component B) into the vial of Aldehyde Standard (Component D) to make a 10 mM aldehyde standard stock solution.

*Note: The unused 10 mM Aldehyde standard stock solution should be divided into single use aliquots and stored at -20°C.*

3.2 Take 100 µL of 10 mM aldehyde standard stock solution (from Step 3.1) to perform 1:10, and 1:3 serial dilutions to get 1000, 300, 100, 30, 10, 3, 1 and 0 µM serial dilutions of aldehyde standard.

3.3 Add serially diluted formaldehyde standards and aldehyde-containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

**Table 1.** Layout of Aldehyde Standards and test samples in a solid black 96-well microplate

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

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

**Table 2.** Reagent composition for each well

Formaldehyde Standards	Blank Control	Test Sample
Serial dilutions*: 50 µL	Assay Buffer: 50 µL	50 µL

*\*Note: Add the serially diluted Aldehyde standards from 0.1 µM to 100 µM into wells from FS1 to FS7 in duplicate.*

**4. Run aldehyde assay:**

4.1 Add 50 µL of AldeLight™ Blue reaction mixture (from Step 2) into each well of formaldehyde standard, blank control, and test samples (see Step 3.4) to make the total aldehyde assay volume of 100 µL/well.

*Note: For a 384-well plate, add 25 µL of test sample and 25 µL of AldeLight™ Blue reaction mixture into each well.*

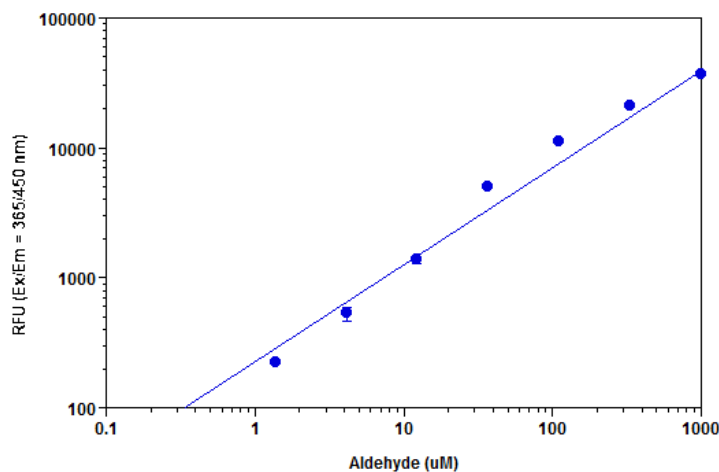
4.2 Incubate the reaction mixture at room temperature for 15 to 30 minutes (protected from light).

4.3 Add 25 µL of Reaction Buffer (Component C) into each well (from Step 4.2).

4.4 Monitor the fluorescence increase at Ex/Em = 365/435 nm using a fluorescence plate reader.

## Data Analysis

The fluorescence in blank wells (with 0  $\mu\text{M}$  Aldehyde Standard and AldeLight™ Blue reaction mixture only) is used as a control, and subtracted from the values of those wells with the formaldehyde reactions. A formaldehyde standard curve is shown in Figure 1.



**Figure 1.** Formaldehyde dose response was measured in a solid black 96-well plate with Amplite™ Fluorimetric Aldehyde Quantitation Kit using a Gemini fluorescence microplate reader (Molecular Devices). As low as 3  $\mu\text{M}$  of aldehyde can be detected with 15 minutes incubation ( $n=3$ ). *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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**Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at [info@aatbio.com](mailto:info@aatbio.com) if you have any questions.**

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