Amplite™ Colorimetric Aldehyde Quantitation Kit *Blue Color*

Catalog number: 10053
Unit size: 200 Tests

Component	Storage	Amount
Component A: AldeView™ Blue	Freeze (<-15 °C), Minimize light exposure	2 bottles
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (25 mL)
Component C: AldeView™ Blue Enhancer	Freeze (<-15 °C), Minimize light exposure	1 bottle (10 mL)
Component D: Aldehyde Standard	Freeze (<-15 °C), Minimize light exposure	1 vial

OVERVIEW

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. Kit 10053 uses a proprietary chromogenic dye that generates a strongly color product upon reacting with an aldehyde. This colorimetric kit provides a sensitive mix-and-read method to detect aldehydes (0.4 uM). 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.

AT A GLANCE

Protocol summary

1. Prepare Aldehyde standards and/or test samples (50 µL)
2. Add 2X AldeView™ Blue working solution (50 µL)
3. Incubate at RT for 20 minutes
4. Add AldeView™ Blue Enhancer (50 µL)
5. Incubate at RT for 20 minutes
6. Monitor absorbance increase at 620 nm

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

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	620 nm
Recommended plate:	Clear bottom

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

PREPARATION OF STANDARD SOLUTION

Aldehyde standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/10053

Take 10 mM Aldehyde standard solution and perform 1:100 in Assay Buffer (Component B) to make 100 µM Aldehyde standard solution (AS7). Take 100 µM Aldehyde standard solution (AS7) and perform 1:2 serial dilutions to get serially diluted Aldehyde standards (AS6 - AS1) with Assay Buffer (Component B).

PREPARATION OF WORKING SOLUTION

Add 5 mL of Assay Buffer (Component B) into one bottle of AldeView™ Blue (Component A) to make 2X AldeView™ Blue working solution.

Note 5 mL of 2X AldeView™ Blue working solution is enough for one plate. 2X AldeView™ Blue working solution is not stable, and best used within 2 hours.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Aldehyde Standards and test samples in a white 96-well microplate with clear bottom. AS= Aldehyde Standards (AS1 - AS7, 1.56 to 100 µM), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
AS1	AS1	...	...
AS2	AS2	...	...
AS3	AS3		
AS4	AS4		
AS5	AS5		
AS6	AS6		
AS7	AS7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
AS1 - AS7	50 µL	Serial Dilutions (1.56 to 100 µM)
BL	50 µL	Assay Buffer
TS	50 µL	test sample

1. Prepare Aldehyde standards (AS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 12.5 µL of reagent per well instead of 50 µL.
2. Add 50 µL of AldeView™ Blue working solution to each well of Aldehyde standard, blank control, and test samples to make the total Aldehyde assay volume of 100 µL/well. For a 384-well plate, add 12.5 µL of AldeView™ Blue working solution into each well instead, for a total volume of 25 µL/well.
3. Incubate the reaction at room temperature for 20 - 30 minutes, protected from light.
4. Add 50 µL of AldeView™ Blue Enhancer (Component C) into each well. For a 384-well plate, add 25 µL of AldeView™ Blue Enhancer into each well.
5. Incubate the reaction at room temperature for 20 minutes, protected from light.

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6. Monitor the absorbance increase with an absorbance plate reader at around 620 to 660 nm (Max at 620 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) 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 Aldehyde 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

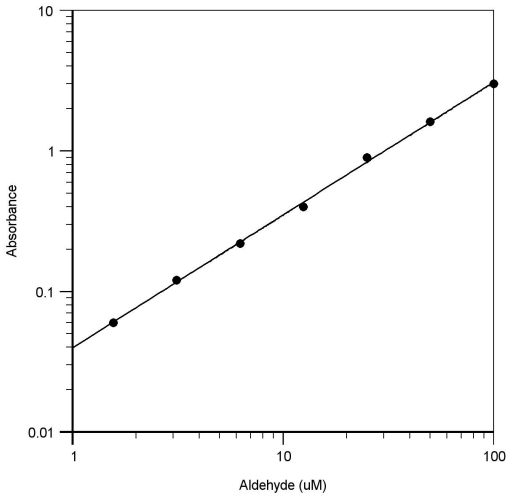

Figure 1. Aldehyde dose response was measured in a white wall/clear bottom 96-well plate with Amplite Colorimetric Aldehyde Quantitation Kit using a SpectraMax microplate reader (Molecular Devices).

DISCLAIMER