Amplite™ Colorimetric Aldehyde Quantitation Kit

Blue Color

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 10053 (200 assays)	Keep at -20 °C Avoid exposure to light	Absorbance microplate readers

Introduction

The formation, reactivity and toxicity of aldehydes resulted 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 aldehydes. Most of the existing aldehyde test methods are based on separations either by the tedious and expensive HPLC-MS or GC-MS.

Our Amplite™ Colorimetric Aldehyde Quantitation Kit uses a proprietary sensor that generates a chromogenic product with an absorbance at 620 nm upon reacting with an aldehyde. This 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 and the signal can be read by an absorbance plate reader at the wavelength between 620 and 660 nm.

Kit Key Features

Broad Application:	Used for quantifying aldehydes in a variety of applications, such as enzyme reactions.
Sensitive:	Detect as little as 0.3 nanomoles of aldehyde in a 100 µL assay volume.
Continuous:	Easily adapted to automation without a separation step.

Kit Components

Components	Amount
Component A: AldeView™ Blue	2 bottles
Component B: Assay Buffer	1 bottle (25 mL)
Component C: AldeView™ Blue Enhancer	1 bottle (10 mL)
Component D: Aldehyde Standard	1 vial

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare Aldehyde standards and/or test samples (50 µL) → Add 2X AldeView™ Blue reaction mixture (50 µL) → Incubate at RT for 20 minutes → Add 50 µL of AldeView™ Blue Enhancer→ Incubate at RT for 20 minutes → Monitor absorbance increase at 620 nm

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

1. Prepare 2X AldeView™ Blue reaction mixture:

Add 5 mL of Assay Buffer (Component B) into one bottle of AldeView™ Blue (Component A) to make AldeView™ Blue reaction mixture.
Note: 5 mL of 2 X AldeView™ Blue reaction mixture is enough for one plate. The reaction mixture is not stable, and best used within 2 hours.

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2. Prepare serial dilutions of aldehyde standard (0 to 100 µM):

2.1 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.

2.2 Take 100 µL of 10 mM aldehyde standard stock solution (from Step 2.1) to perform 1:100, and 1:2 serial dilutions to get 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0 µM serial dilutions with Assay Buffer (Component B).

2.3 Add serially diluted aldehyde standards and aldehyde-containing test samples into a white 96-well microplate with clear bottom as described in Tables 1 and 2.

Table 1. Layout of Aldehyde Standards and test samples in a white 96-well microplate with clear bottom

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

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

Table 2. Reagent composition for each well

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

**Note: Add the serially diluted aldehyde standards from 1.56 µM to 100 µM into wells from AS1 to AS7 in duplicate.*

3. Run aldehyde assay:

3.1 Add 50 µL of 2X AldeView™ Blue reaction mixture (from Step 1) into each well of aldehyde standard, blank control, and test samples (see Step 2.3) to make the total aldehyde assay volume of 100 µL/well.
Note: For a 384-well plate, add 12.5 µL of test sample and 12.5 µL of 2X AldeView™ Blue reaction mixture into each well.

3.2 Incubate the reaction mixture at room temperature for 20-30 minutes (protected from light).

3.3 Add 50 µL of AldeView™ Blue Enhancer (Component C) into each well.
Note: For a 384-well plate, add 25 µL of AldeView™ Blue Enhancer into each well.

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

Data Analysis

The absorbance in blank wells (with 0 µM Aldehyde Standard) is used as a control, and is subtracted from the values of those wells with the aldehyde reactions. An aldehyde standard curve is shown in Figure 1.
Note: The absorbance background increases with time, thus it is important to subtract the absorbance value of the blank wells for each data point.

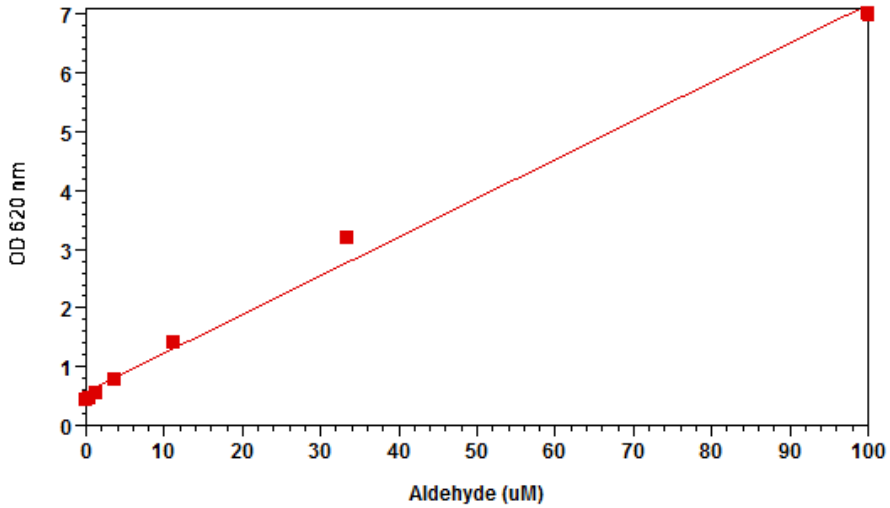

Figure 1. Aldehyde dose response was measured in a white wall/clear bottom 96-well plate with Amplite™ Colorimetric Aldehyde Quantitation Kit using a SpectraMax microplate reader (Molecular Devices). As low as ~3 µM of aldehyde can be detected with 30 minutes incubation (n=3).

References

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