Amplite™ Colorimetric Aldehyde Quantitation Kit

Catalog number: 10051
Unit size: 200 Tests

Component	Storage	Amount
Component A: AldeView™ Yellow	Freeze (< -15 °C), Minimize light exposure	2 bottles
Component B: Assay Solution	Freeze (< -15 °C), Minimize light exposure	1 bottle (10 mL)
Component C: Aldehyde Standard	Freeze (< -15 °C), Minimize light exposure	1 vial
Component D: Dilution Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (20 mL)

OVERVIEW

Rapid and accurate measurement of aldehydes is important task for biological research, food industry, chemical research and environmental pollution surveillance. There are few reagents or assay kits available for quantifying the amount of aldehydes. Most of existing aldehyde test methods is based on separations either by the tedious and expensive HPLC-MS or GC-MS. Our Amplite™ Colorimetric Aldehyde Quantitation kit uses a proprietary dye that generates a chromogenic product upon reacting with an aldehyde. The kit provides a sensitive, one-step colorimetric method to detect as little as 1 nanomole of aldehyde in a 100 µL assay volume (10 µM; Figure 1). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. Its signal can be easily read by absorbance microplate reader at 550 nm.

AT A GLANCE

Protocol Summary

1. Prepare Aldehyde standards and/or test samples (50 µL)
2. Add 2X AldeView™ Yellow working solution (50 µL)
3. Incubate at room temperature for 30 to 60 minutes
4. Monitor absorbance increase at 550 nm

Important Thaw all the kit components to room temperature before starting the experiment. Assay solution (Component B) is potentially hazardous. Wear gloves when handling it.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	550 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.

Aldehyde standard solution (10 mM)
Add 1 mL of Dilution Buffer (Component D) into the vial of Aldehyde Standard (Component C) to make a 10 mM aldehyde standard solution.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/10051

Aldehyde standard
Take 10 mM Aldehyde standard solution and perform 1:10 dilution to get 1000 uM Aldehyde standard solution (AS7). Then perform 1:3 serial dilutions to get remaining serial dilutions of aldehyde standard (AS6 - AS1).

PREPARATION OF WORKING SOLUTION

Add 5 mL of Assay Solution (Component B) into the bottle of AldeView™ Yellow (Component A), and mix well.

Note 5 mL of the 2X AldeView™ Yellow reaction mixture is enough for 1 plate. The reaction mixture is not stable. Use within 2 hours.

Note Assay solution (Component B) is potentially hazardous. Wear gloves when handling it.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Aldehyde standards and test samples in a white/clear bottom 96-well microplate. AS= Aldehyde Standards (AS1 - AS7, 1 to 1000 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 Dilution (1 to 1000 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 25 µL of reagent per well instead of 50 µL. *Note:* Both BSA and Tween 20 will interfere with the assay, use less than 0.001% BSA and 0.01% Tween 20 in the samples.

   Note If the aldehyde-containing samples are from enzyme reactions such as fructose-1,6-bisphosphate with fructose-1,6-bisphosphate aldolase, prepare 50 µL of enzyme reaction (25 µL for a 384-well plate) as desired. Incubate the enzyme reaction at 37 °C for at least 1 hour. The components of enzyme reaction should be optimized as needed (e.g. an optimized buffer system might be required for a specific enzyme reaction). In most cases, Dilution Buffer (Component D) can also be used for running enzyme reaction if you do not have an optimized enzyme buffer.

2. Add 50 µL of AldeView™ Yellow 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 25 µL of AldeView™ Yellow working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction mixture at room temperature for 30 to 60 minutes, protected from light.

4. Monitor the absorbance increase with an absorbance plate reader at 550 nm.

Note Different concentrations of the aldehyde might form different colors with AldeView™ Yellow.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (OD 550 nm) 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 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

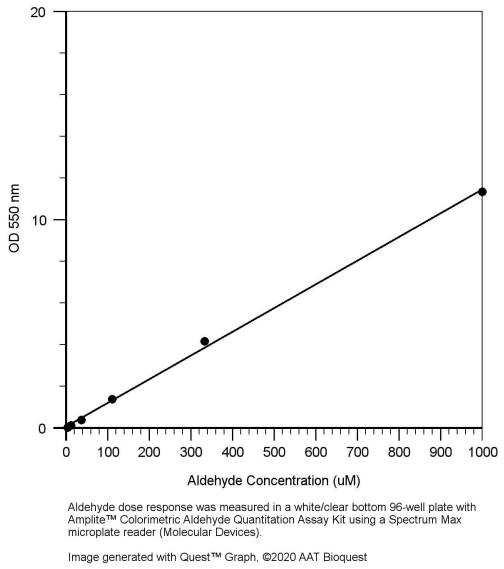

Figure 1. Aldehyde dose response was measured in a white/clear bottom 96-well plate with Amplite™ Colorimetric Aldehyde Quantitation Assay Kit using a Spectrum Max microplate reader (Molecular Devices).

DISCLAIMER

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