Amplite™ Colorimetric Aldehyde Quantitation Kit

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 10051 (200 assays)	Keep at -20 °C Avoid exposure to moisture and light	Absorbance microplate readers

Introduction

Rapid and accurate measurement of aldehydes 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 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. Our Amplite™ Colorimetric Aldehyde Qutitation 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 nanoomole 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 without a separation step. Its signal can be easily read by an absorbance microplate reader at 405 or 550 nm.

Kit Key Features

Broad Application:	Can be used for quantifying aldehydes in a variety of applications such as carbohydrate, lipid chemistry, as well as enzyme reactions.
Sensitive:	Detect as low as 1 nanomole of aldehyde.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: AldeView™ Yellow	2 bottles
Component B: Assay Solution	1 bottle (10 mL)
Component C: Aldehyde Standard	1 vial
Component D: Dilution Buffer	1 bottle (20 mL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare Enzyme reaction (50 µL) → Add 2X AldeView™ Yellow reaction mixture (50 µL) → Incubate at room temperature for 30 to 60minutes → Read Absorbence at 405 or 550 nm

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

1. Prepare 2X AldeView™ Yellow reaction mixture:

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

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

Note 2: Assay solution (Component B) is potentially hazardous. Wear gloves when handling it.

2. Prepare serial aldehyde standard (0 to 1 mM) solutions:

2.1 Add 1 mL of Dilution Buffer (Component D) into the vial of Aldehyde Standard (Component C) to make 10 mM 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 solution (from Step 2.1) to perform 1:10, and 1:3 serial dilutions to get 1000, 300, 100, 30, 10, 3, 1, 0.3, and 0 µM standard aldehyde solutions.

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

Note 1: Both BSA and Tween 20 will interfere the assay, use less than 0.001% BSA and 0.01% Tween 20 in the samples.

Note 2: If the aldehyde-containing samples are from the enzyme reaction such as fructose-1,6-bisphosphae with fructose-1,6-bisphosphae aldolas, prepare 50µL of enzyme reaction (25 µL for 384-well plates) 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).

Note 3: In most cases, Dilution Buffer (Component D) can also be used for running enzyme reaction if you do not have an optimized enzyme buffer.

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

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

**Note: Add the serially diluted Aldehyde standards from 0.3 µM to1000 µM into wells from AS1 to AS7 in duplicate.*

3. Run aldehyde assay:

3.1 Add 50 µL of 2X AldeView™ Yellow reaction mixtures (from Step 1) to each well of the 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 25 µL of sample and 25 µL of aldehyde reaction mixture into each well.

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

3.3 Monitor the absorbance increase at 405 or 550 nm using an absorbance plate reader.

Note: Different concentrations of the aldehyde might form different colors with AldeView™ Yellow. At lower concentration, the absorbance at 405 nm gives the best result. However, at higher concentration, the absorbance tends to shift to 630 nm.

Data Analysis

The absorbance in blank wells (with 0 aldehyde standards and 2X AldeView™ Yellow reaction mixture only) is used as a control, and is subtracted from the values for 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 intensity value of the blank wells for each data point.

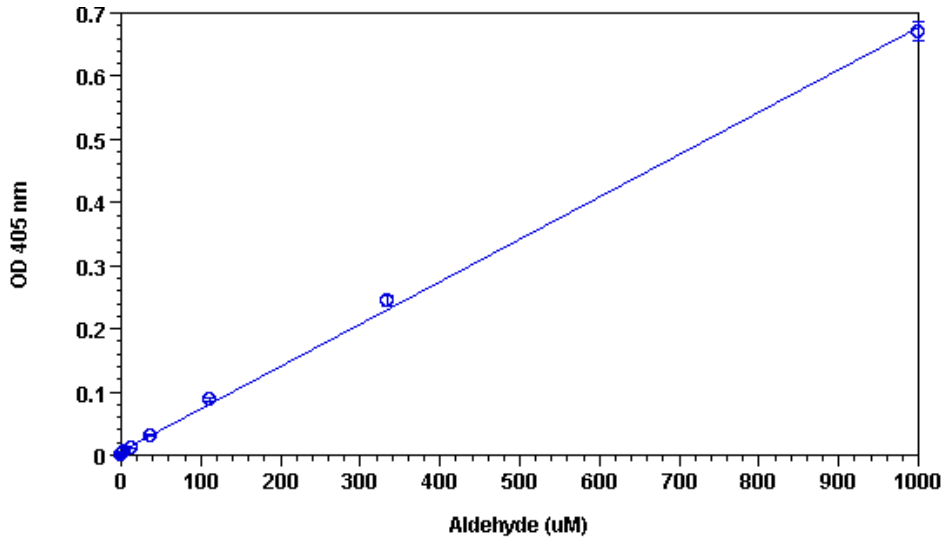

Figure 1. Aldehyde dose response was measured in a 96-well black plate with Amplite™ Colorimetric Aldehyde Quantitation Assay Kit using a Spectrum Max microplate reader (Molecular Devices). As low as 10 µM (1 nanomol/well) of aldehyde can be detected with 30 minutes incubation time (n=3).

References

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