Amplite™ Colorimetric Aldehyde Quantitation Kit

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

Introduction

Very reactive aldehydes, namely 4-hydroxyalkenals, were first shown to be formed in autoxidizing chemical systems. It was subsequently shown that 4-hydroxyalkenals, particularly 4-hydroxynonenal, were formed in substantial amounts under biological conditions, i.e. during the peroxidation of lipids of liver microsomes incubated in the NADPH-Fe system. Many other aldehydes were also identified in peroxidizing liver microsomes or hepatocytes, e.g., alkanals, alk-2-enals, and 4-hydroxyalkenals.

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 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). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and readily adapted to automation without a separation step. Its signal can be easily read with an absorbance microplate reader at 405 or 550 nm. This kit has been used for monitoring activities of oxidases that convert an amino group to an aldehyde group.

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 60 minutes → Monitor absorbance increase 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 hours.
Note 2: Assay solution (Component B) is potentially hazardous. Wear gloves when handling it.

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

2.1 Add 1 mL of Dilution Buffer (Component D) into the vial of Aldehyde Standard (Component C) to make a 10 mM aldehyde standard stock solution.
Note: The unused 10 mM Aldehyde standard stock 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 1:10, and 1:3 serial dilutions to get 1000, 300, 100, 30, 10, 3, 1, and 0 µM serial dilutions of aldehyde standard.

2.3 Add serial dilutions of aldehyde standard 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-bisphosphate with fructose-1,6-bisphosphate aldolase, prepare 50µL 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

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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 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 serial dilutions of aldehyde standard from 1 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) into 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 with an absorbance plate reader at 405 or 550 nm.
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 550 nm.

Data Analysis

The absorbance in blank wells (with 0 µM aldehyde standard 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.*

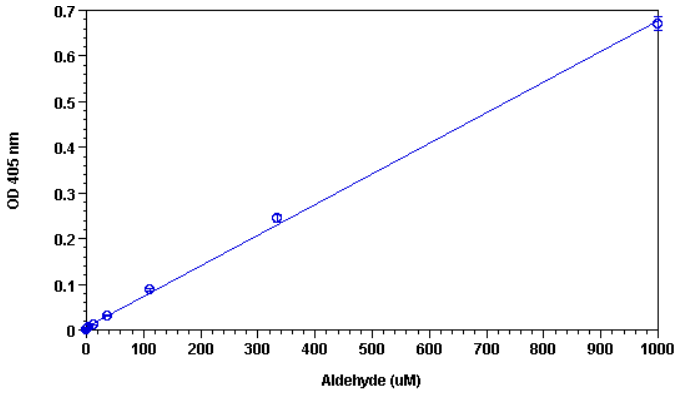

Figure 1. Aldehyde dose response was measured in a white/clear bottom 96-well plate with Amplite™ Colorimetric Aldehyde Quantitation Assay Kit using a Spectrum Max microplate reader (Molecular Devices). As low as 10 µM (1 nanomol/well) of aldehyde can be detected with 30 minutes incubation (n=3).

References

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3. Integrated self-assembling drug delivery system possessing dual responsive and active targeting for orthotopic ovarian cancer theranostics
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