ab112113

Aldehyde Quantification Assay Kit (Colorimetric)

Instructions for Use

For detecting quantifying aldehyde in a variety of applications using a one step colorimetric method

View kit datasheet: www.abcam.com/ab112113
(use www.abcam.cn/ab112113 for China, or www.abcam.co.jp/ab112113 for Japan)

This product is for research use only and is not intended for diagnostic use.

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1. 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.

ab112113 Aldehyde Quantitation Kit (Colorimetric) uses a proprietary dye that generates a chromogenic product upon reacting with an aldehyde. Most of the existing aldehyde test methods are based on separations either by the tedious and expensive HPLC-MS or GC-MS. ab112113 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 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.

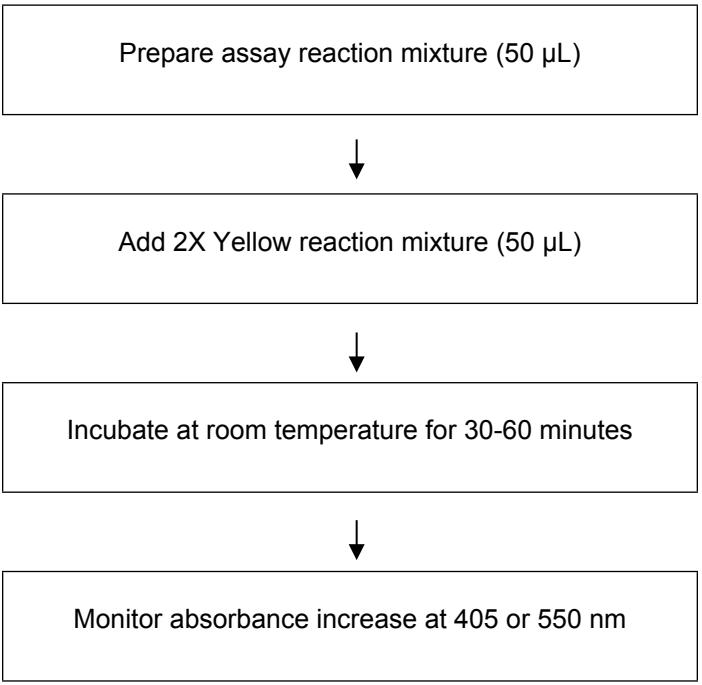
- **Continuous:** Easily adapted to automation without a separation step.

- **Convenient:** Formulated to have minimal hands-on time. No wash is required.

- **Non-Radioactive:** No special requirements for waste treatment.

2. Protocol Summary

Summary for One 96-well Plate



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

3. Kit Contents

Components	Amount
Component A: Yellow Indicator	2 x bottles
Component B: Assay Buffer	1 x 10 mL
Component C: Aldehyde Standard	1 vial
Component D: Dilution Buffer	1 x 20 mL

4. Storage and Handling

Keep at -20°C. Avoid exposure to moisture and light.

5. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Prepare 2X Yellow Reaction Mixture

Add 5 mL of Assay Buffer (Component B) into the bottle of Yellow Indicator (Component A), and mix well.

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

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

B. Prepare serial dilutions of Aldehyde Standard

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 solution should be divided into single use aliquots and stored at -20 °C.

2. Take 100 µL of 10 mM aldehyde standard stock solution (from Step 1) to perform 1:10, and 1:3 serial dilutions to get 1000, 300, 100, 30, 10, 3, 1, 0.3, and 0 µM serial dilutions of aldehyde standard.

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 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).

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.

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

Table 1. Layout of Aldehyde standards and test samples in a white/clear 96-well microplate.
Note: A*S= Aldehyde Standards, BL=Blank Control, TS=Test Samples.*

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

Table 2. Reagent composition for each well.
**Note: Add the serially diluted calcium standards from 0.3 µM to 1000 µM into wells from AS1 to AS7 in duplicate.*

C. Run Aldehyde Assay:

1. Add 50 µL of 2X Yellow reaction mixture (from Step A) into each well of the aldehyde standard, blank control, and test samples (see Step 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.

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

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 Yellow Indicator. At lower concentration, the absorbance at 405 nm gives the best result. However, at higher concentration, the absorbance tends to shift to 550 nm.

6. Data Analysis

The absorbance in blank wells (with 0 aldehyde standards and 2X 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.

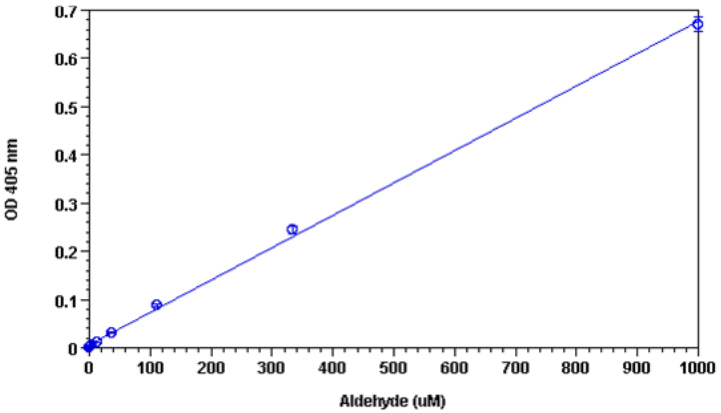


Figure 1. Aldehyde dose response was measured in a 96-well black plate with ab112113 using a microplate reader. As low as 10 µM (1 nmol/well) of aldehyde can be detected with 30 minutes incubation (n=3). Standard curve read at 405 nm.

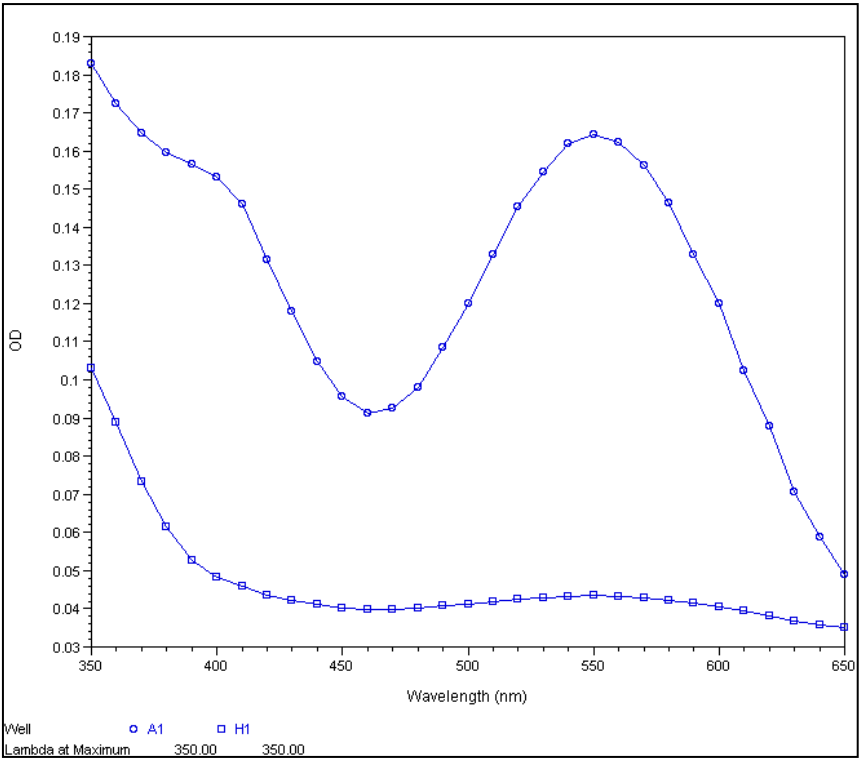


Figure 2. Aldehyde Spectrum. The absorbance spectrum of glyceraldehyde, after aldehyde reaction with Yellow Indicator. The squares represent buffer only. The circles represent glyceraldehdyde. The spectrum shape doesn't change with concentration, but the intensities (at both 405 nm and 550 nm) increase with glyceraldehdyde concentration.

7. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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