ab138882

Aldehyde Quantification Assay Kit (Fluorometric)

Instructions for Use

For quantifying aldehydes, at higher pH, in a variety of applications, such as enzyme reactions.

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

Version 2 Last updated 31 January 2019

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1. Introduction

The formation, reactivity and toxicity of aldehydes originating 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 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.

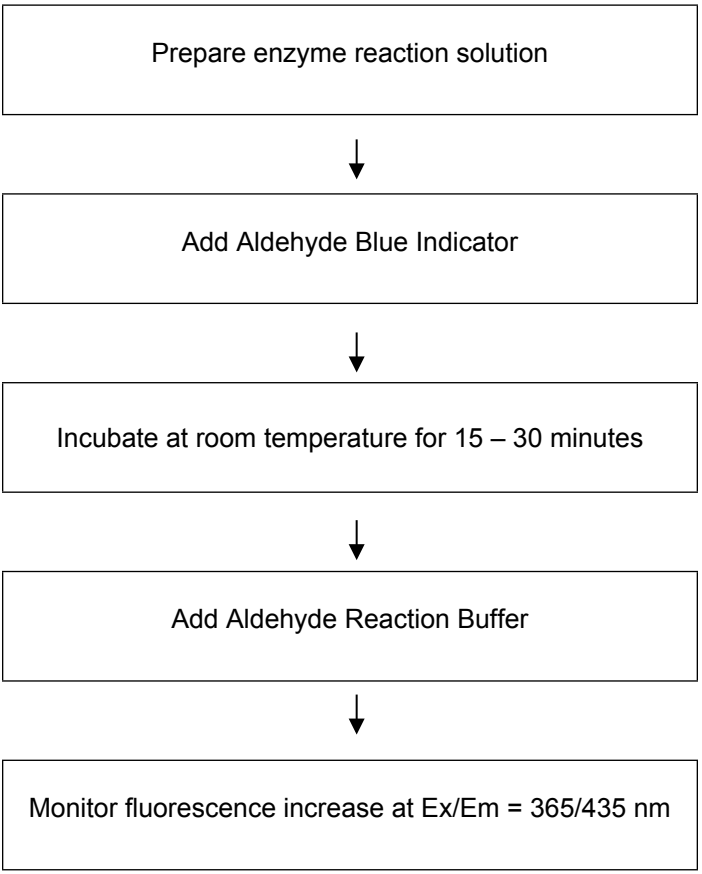
ab138882 is used for quantifying aldehydes at higher pH by using a proprietary fluorogenic dye that generates a strongly fluorescent product upon reacting with an aldehyde. This fluorimetric kit provides a sensitive mix-and-read method to detect as little as 0.3 nanomole of aldehyde in a 100 µl assay 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. Its signal can be read by a fluorescence microplate reader at Ex/Em = 365/435 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 nanomole of aldehyde in a 100 µl assay volume.
- **Continuous:** Easily adapted to automation without a separation step.
- **Convenient:** Formulated to have minimal hands-on time.
- **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
Aldehyde Blue Indicator	1 vial
Assay Buffer	1 bottle (30 ml)
Aldehyde Reaction Buffer	1 vial (6 ml)
Aldehyde Standard	1 vial
DMSO	1 vial (100 µl)

4. Storage and Handling

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

5. Additional Materials Required

- 96 or 384-well microplates: Solid black microplates
- Fluorescence microplate reader

6. Assay Protocol

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

A. Preparation of stock solution

1. Preparation of 250X Aldehyde Blue Indicator stock solution: Add 40 µl of DMSO in the vial of Aldehyde Blue Indicator to make Aldehyde Blue Indicator stock solution. *Note: The unused Aldehyde Blue Indicator stock solution should be divided into single use aliquots, and stored at -20 °C.*

B. Preparation of Assay Reaction Mixture

1. Preparation of 250X Aldehyde Blue Indicator assay reaction mixture: Add 20 µl of 250X Aldehyde Blue Indicator stock solution into 5 ml of Assay Buffer and mix well. *Note: 5 ml of Aldehyde Blue Indicator assay reaction mixture is enough for one plate. The mixture is not stable and should be used within 2 hours.*

C. Preparation of Serial Dilutions of Aldehyde Standard (0 to 1 mM)

1. Add 1 ml of Assay Buffer into the vial of Aldehyde Standard to make 10 mM Aldehyde Standard stock solution. *Note: The unused 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 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.

3. Add serially diluted aldehyde standards and aldehyde-containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

Table 1. Layout of Aldehyde Standards and test samples in a solid black 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 1 µM to 1000 µM into wells from AS1 to AS7 in duplicate*

D. Run Aldehyde Assay:

1. Add 50 µl of Aldehyde Blue Indicator Assay Reaction Mixture into each well of aldehyde standard, blank control, and test samples make the total aldehyde assay volume of 100 µl/well.

   Note: For a 384-well plate, add 25 µl of test sample and 25 µl of Aldehyde Blue Indicator Assay Reaction Mixture into each well.

2. Incubate the Aldehyde Blue Indicator Assay Reaction Mixture at room temperature for 15-30 minutes, protected from light.

3. Add 25 µl of Reaction Buffer into each well.

4. Monitor the fluorescence increase at Ex/Em = 365/435 nm using a fluorescence plate reader.

7. Data Analysis

The fluorescence in blank wells (0 µM Aldehyde Standard and Aldehyde Blue Indicator reaction mixture only) is used as a control, and subtracted from the values of those wells with the aldehyde reactions.

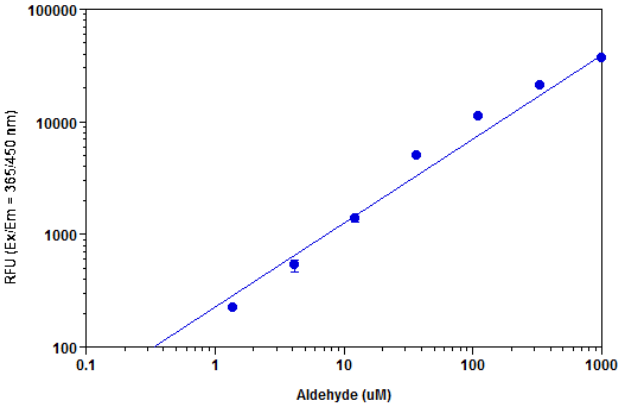


Figure 1. Aldehyde dose response was measured in a solid black 96-well plate with ab138882 using a fluorescence microplate reader. As low as 3 µM of aldehyde can be detected with 15 minutes incubation (n=3). *Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.*

8. 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)

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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