Aldehyde Assay Kit

Catalog Number KA4125
200 assays
Version: 02

Intended for research use only

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Introduction

Intended Use

Aldehyde Assay Kit is a colorimetric assay for the quantification of aldehydes in a variety of applications with absorbance at 405 nm or 550 nm.

Background

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.

Principle of the Assay

Most of the existing aldehyde test methods are based on separations either by the tedious and expensive HPLC-MS or GC-MS. Our Aldehyde Assay Kit uses a 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.

✓ 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.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component A: AldeView Yellow</td>
<td>2 bottles</td>
</tr>
<tr>
<td>Component B: Assay Solution</td>
<td>10 mL</td>
</tr>
<tr>
<td>Component C: Aldehyde Standard</td>
<td>1 vial</td>
</tr>
<tr>
<td>Component D: Dilution Buffer</td>
<td>20 mL</td>
</tr>
</tbody>
</table>

Storage Instruction

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

Precautions for Use

✓ This kit is For Research Use Only.
✓ Avoid moisture and light.
Assay Protocol

Reagent Preparation

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

✔ Prepare 2X AldeView Yellow reaction mixture:
1. 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.
2. Assay solution (Component B) is potentially hazardous. Wear gloves when handling it.

Sample Preparation

✔ Prepare serial dilutions of aldehyde standard (0 to 1 mM):
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 Step1) 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 Plate Layout and Table 1.

Table 1  Reagent composition for each well

<table>
<thead>
<tr>
<th>Aldehyde Standard</th>
<th>Blank Control</th>
<th>Test Sample</th>
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<tbody>
<tr>
<td>Serial Dilutions* (50 μL)</td>
<td>Assay Buffer: 50 μL</td>
<td>50 μL</td>
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</table>

* Add the serial dilutions of aldehyde standard from 0.3 μM to1000 μM into wells from AS1 to AS7 in duplicate.

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.
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).

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.

**Assay Procedure**

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 3 of Sample Preparation) 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 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.

**✓ Summary**

1. Prepare enzyme reaction (50 μL)
2. Add 2X AldeView Yellow reaction mixture (50 μL)
3. Incubate at room temperature for 30 to 60 minutes
4. Monitor absorbance increase at 405 or 550 nm
Data Analysis

Calculation of Results

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 Aldehyde 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).
Resources

References


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<th>A</th>
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AS= Aldehyde Standards
BL= Blank Control
TS= Test Samples