Acetaldehyde Assay Kit (Colorimetric)

LS-K276-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Introduction

ACETALDEHYDE (CH_3CHO) is one of the most widely occurring aldehydes in nature and is commonly used in industry. Acetaldehyde, a metabolic byproduct of ethanol in the liver, is toxic to the human body and is rapidly converted to the less harmful acetic acid by the enzyme aldehyde dehydrogenase. People with a deficiency of aldehyde dehydrogenase accumulate acetaldehyde when consuming alcohol and this accumulation results in facial and body flushing often referred to as “Asian flush syndrome.” Buildup of acetaldehyde has also been associated with the effects of hangovers from alcohol consumption. Although classified as a carcinogen, acetaldehyde is naturally found in many foods and beverages such as ripe fruit, coffee, and wine.

LSBio’s colorimetric acetaldehyde assay kit is based on aldehyde dehydrogenase catalyzed oxidation of acetaldehyde, in which the formed NADH reduces a formazan reagent. The intensity of the product color, measured at 565 nm, is directly proportional to the acetaldehyde concentration in the sample.

Key Features

- Fast and sensitive. Linear detection range (20 µL sample): 2 µM to 2mM acetaldehyde in 96-well plate assay.
- Convenient. The procedure involves adding a single working reagent, and reading the absorbance after 30 minutes. Room temperature assay. No 37°C heater is needed.
- High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Applications

- Acetaldehyde in biological samples (e.g. plasma, serum, urine, tissue and culture media.) and food/beverage samples (e.g. wine, coffee, and juice)

Components

Component	K276-100
	100 Tests
Assay Buffer	10 mL
NAD/MTT	1 mL
Standard	100 µL
Enzyme A	120 µL
Enzyme B	120 µL

Materials Not Supplied

Pipetting devices and accessories (e.g. multi-channel pipette), clear flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes and plate reader.

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Storage

The kit is shipped on ice. Store all kit components at -20 °C.

Assay Procedure

Sample Preparation: clear and slightly colored samples can be assayed directly. It is prudent to test several dilutions to determine an optimal dilution factor n.

Biological fluid samples (e.g. urine & serum) can be assayed directly after centrifuging to remove any particulates. Appropriate dilution in distilled water may be required.

Reagent Preparation: equilibrate Assay Buffer and NAD/MTT solution to room temperature. Briefly centrifuge tubes before use. Keep Enzymes and Standard on ice.

Reaction Preparation:

1. Standards. Prepare 1 mL 2 mM Premix by mixing 5 µL of the Standard (400 mM) and 995 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	2 mM Premix + H_2O	Acetaldehyde (mM)
1	100 µL + 0 µL	2.0
2	60 µL + 40 µL	1.2
3	30 µL + 70 µL	0.6
4	0 µL + 100 µL	0

2. Transfer 20 µL standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 20 µL of each sample in duplicate into separatewells (one well as "Sample" and one well as "Sample Blank").
3. Prepare sufficient Working Reagent (WR) for all the Standards and “Sample” wells by mixing, for each well: 75 µL Assay Buffer, 8 µL NAD/MTT, 1 µL Enzyme A, and 1 µL Enzyme B. Prepare sufficient Blank Working Reagent (BWR) for the “Sample Blank” wells by mixing, for each well: 75 µL Assay Buffer, 8 µL NAD/MTT, and 1 µL Enzyme B. (i.e. no Enzyme A). Add 80 µL WR to the Standards and the “Sample” wells. Add 80 µL BWR to the “Sample Blank” wells. Tap plate to mix briefly and thoroughly. Incubate 30 minutes at room temperature.
4. Read optical density at 565 nm (520-600 nm).

Calculations:

Subtract the blank value (#4) from the standard values and plot the DOD against standard concentrations. Determine the slope and calculate the acetaldehyde concentration of Sample,

$$[\text{Acetaldehyde}] = \frac{OD_S - OD_{SB}}{\text{Slope } (mM^{-1})} \times n \quad (mM)$$

where ODS and ODSB are optical density readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.

Note: if the sample OD value is higher than OD for the 2 mM acetaldehyde standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

Conversions: 1 mM acetaldehyde equals 4.4 mg/dL, or 44 ppm.

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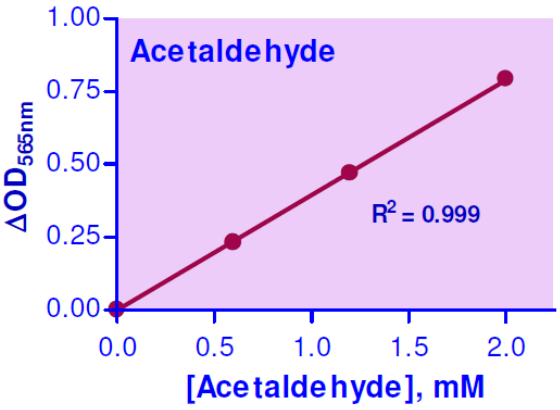
Sample Data



Standard Curve in 96-well plate assay in water.

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