Acetaldehyde Assay Kit (Fluorometric)

LS-K275-100 (100 Tests) • **Store at -20°C**

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. This fluorometric acetaldehyde assay is based on aldehyde dehydrogenase catalyzed oxidation of acetaldehyde, in which the generated NADH reduces a probe making it fluorescent. The fluorescence intensity of the product measured at $\lambda_{ex/em}$ = 530/585 nm is directly proportional to acetaldehyde concentration in the sample.

Key Features

- Fast and sensitive. Linear detection range (50µL sample): 0.5 µM to 60 µM acetaldehyde in 96-well plate assay.
- High-throughput. The procedure involves adding a single working reagent, and reading the absorbance after 30 minutes. Room temperature assay. No 37°C heater is needed.
- Convenient. 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), or food/beverage samples (e.g. wine, coffee, and juice).

Components

Component	K275-100
	100 Tests
Assay Buffer	10 mL
NAD Solution	1 mL
Probe	750 µL
Enzyme A	120 µL
Enzyme B	120 µL
Standard	100 µL

Materials Not Supplied

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

Storage

The kit is shipped on ice. Store components at -20°C upon receiving. Standard may be stored at -20°C to 4°C. Shelf life: 6 months after receipt.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

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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 solution, and Probe to room temperature. Briefly centrifuge tubes before use. Keep Enzymes and Standard on ice.

Procedure

1. Standards. Prepare 1 mL 4 mM Acetaldehyde by mixing 10 µL of the Standard (400 mM) and 990 µL distilled water. Prepare 1 mL of 60 µM Premix by mixing 15 µL 4 mM Acetaldehyde with 985 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	60 µM Premix + H_2O	Acetaldehyde (µM)
1	100 µL + 0 µL	60
2	60 µL + 40 µL	36
3	30 µL + 70 µL	18
4	0 µL + 100 µL	0

   Note: Prepare 60 µM Premix and Standards fresh for each assay.

2. Transfer 50 µL standards into separate wells of a black, flat-bottom 96-well plate. Transfer 50 µL of each sample in duplicate into separate wells (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: 40 µL Assay Buffer, 8 µL NAD Solution, 5 µL Probe, 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: 45 µL Assay Buffer, 8 µL NAD Solution, 5 µL Probe, and 1 µL Enzyme B. (i.e. no Enzyme A).

   Add 50 µL WR to the Standards and the “Sample” wells. Add 50 µL BWR to the “Sample Blank” wells. Tap plate to mix briefly and thoroughly. Incubate 30 minutes at room temperature.

4. Read fluorescence at $\lambda_{ex/em}$ = 530/585 nm.

Calculations

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

$$[\text{Acetaldehyde}] = \frac{F_S - F_{SB}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

F_S and F_{SB} are fluorescence readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.

Note: if the sample fluorescence value is higher than fluorescence for the 60 µM acetaldehyde standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

Conversions: 1 µM acetaldehyde equals 4.4 µg/L, or 44 ppb.

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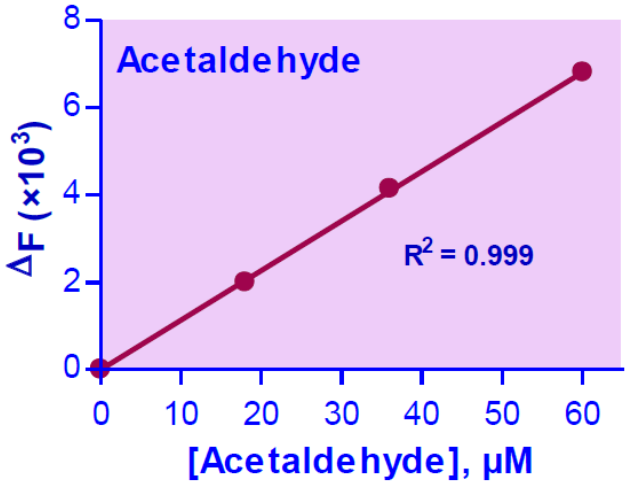
Sample Data



Standard Curve in 96-well plate assay in water

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