Technical Bulletin

Acetaldehyde Assay Kit

Catalogue number MAK321

Product Description

Acetaldehyde is one of the most widely occurring aldehydes in nature and commonly used in industry. The metabolic byproduct of ethanol in the liver, acetaldehyde is toxic to the human body and 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.

The acetaldehyde assay is based on the 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 λex = 530 nm/λem = 585 nm is directly proportional to acetaldehyde concentration in the sample.

The Acetaldehyde Assay Kit can be used to determine acetaldehyde in biological samples (e.g., plasma, serum, urine, tissue, and culture media.) or food/beverage samples (e.g., wine, coffee, and juice).

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalog Number MAK321A
- NAD Solution 1 mL
  Catalog Number MAK321B
- Probe 750 µL
  Catalog Number MAK321C
- Enzyme A 120 µL
  Catalog Number MAK321D
- Enzyme B 120 µL
  Catalog Number MAK321E
- Acetaldehyde Standard, 3 M 100 µL
  Catalog Number MAK321F

Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Fluorescence plate reader
- Black flat-bottom 96 well plates
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. Store components at -20 °C upon receipt. Standard may be stored at -20 °C to 4 °C.

Preparation Instructions

Reagent Preparation

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

Procedure

Use ultrapure water for the preparation of all Standards and Samples.

Acetaldehyde Standards

Prepare 1 mL of 15 mM Standard solution by mixing 5 µL of the Acetaldehyde Standard (3 M) and 995 µL of ultrapure water. Prepare 1 mL of 60 µM Standard solution by mixing 4 µL of 15 mM Standard solution with 996 µL of ultrapure water. Prepare standards in 1.5 mL centrifuge tubes as described in Table 1.

Note: Prepare 60 µM Standard solution and individual Standards fresh for each assay.

Table 1.
Preparation of Acetaldehyde Standards

Tube	60 µM standard	Ultrapure water	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

Transfer 50 µL of each Standard into separate wells of a black, flat-bottom 96 well plate.

Sample Preparation

Clear and slightly colored Samples can be assayed directly. Biological fluid Samples (e.g., urine and serum) can be assayed directly after centrifuging to remove any particulates.

It is prudent to test several dilutions to determine an optimal dilution factor (n). Appropriate dilution in ultrapure water may be required.

Transfer 50 µL of each sample in duplicate into separate wells (one well as "Sample" and one well as "Sample Blank").

Assay Reaction

1. Set up the Master Reaction Mixes according to the scheme in Table 2. 50 µL of the appropriate Master Reaction Mix is required for all wells.

Table 2.
Master Reaction Mixes

Reagent	Standards and Samples Volume	Sample Blank Volume
Assay Buffer	40 µL	41 µL
NAD Solution	8 µL	8 µL
Probe	5 µL	5 µL
Enzyme A	1 µL	–
Enzyme B	1 µL	1 µL

2. Add 50 µL of the appropriate Master Reaction Mix to the Standards, the "Sample", and the "Sample Blank" wells.
3. Tap plate to mix briefly and thoroughly, then incubate for 30 minutes at room temperature. Read fluorescence at λ_{ex} = 530 nm/λ_{em} = 585 nm.

Results

Subtract the blank value (standard tube 4) from the values of the other standards. Plot the ΔF against standard concentrations and determine the slope of the standard curve. Calculate the acetaldehyde concentration of Sample(s):

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

Where:
F_S = fluorescence reading of the Sample
F_{SB} = fluorescence reading of the Sample Blank
n = the sample dilution factor
Note: If the sample fluorescence value is higher than fluorescence for the 60 µM acetaldehyde standard, dilute sample in ultrapure water and repeat the assay.

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

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