Technical Bulletin

Acetaldehyde Assay Kit

Catalog Number MAK434

Product Description

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.

The Acetaldehyde Assay Kit is based on the aldehyde dehydrogenase catalyzed oxidation of acetaldehyde with the reduction of NAD to NADH. The formed NADH subsequently reduces MTT, producing a colored formazan compound. The intensity of the end product color, measured at 565 nm, is directly proportional to the acetaldehyde concentration in the sample.

The linear detection range of acetaldehyde by the kit for a 20 µL sample is 2 µM to 2 mM. The kit is suitable for acetaldehyde determination in biological samples (e.g., plasma, serum, urine, tissue and culture media) and food/beverage samples (e.g., wine, coffee, and juice).

Components

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

- Assay Buffer 10 mL
  Catalog Number MAK434A
- Enzyme A 120 µL
  Catalog Number MAK434B
- NAD/MTT Solution 1 mL
  Catalog Number MAK434C
- Enzyme B 120 µL
  Catalog Number MAK434D
- 3 M Standard 100 µL
  Catalog Number MAK434E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 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 wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Assay Buffer and NAD/MTT Solution: Equilibrate to room temperature prior to use.

Enzyme A and Enzyme B: Keep enzymes on ice during procedure.

3M Standard: Equilibrate to room temperature **immediately** prior to use. Return to -20 °C within 30 minutes of thawing.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

1. Clear and slightly colored samples can be assayed directly. For unknown samples, testing different dilutions of Sample to ensure the readings are within the standard curve range is recommended.
2. Biological fluid samples (e.g., urine or serum) can be assayed directly after centrifuging to remove any particulates. Appropriate dilution in purified water may be required to ensure the sample reading is within the standard curve.
3. Transfer 20 µL of each sample in duplicate into separate wells of a clear, flat-bottom 96-well plate (one well as "Sample" and one well as "Sample Blank").

Working Reagents

Mix enough reagents for the number of assays to be performed. For each Standard and Sample well, prepare 85 µL of Working Reagent. For each Sample Blank well, prepare 84 µL of Blank Working Reagent. Prepare Working Reagents according to Table 1.

Table 1.
Preparation of Working Reagents

Reagent	Working Reagent	Blank Working Reagent
Assay Buffer	75 µL	75 µL
NAD/MTT Solution	8 µL	8 µL
Enzyme A	1 µL	-
Enzyme B	1 µL	1 µL

Standard Curve Preparation

1. Make standards fresh and assay within 10 minutes of preparation.
2. Prepare a 30 mM Acetaldehyde Standard by mixing 5 µL of the 3 M Standard with 495 µL of purified water.
3. Further dilute the 30 mM Acetaldehyde Standard to 2 mM by mixing 20 µL of the 30 mM Acetaldehyde standard with 280 µL of purified water.
4. Prepare Acetaldehyde Standards in 1.5 mL microcentrifuge tubes according to Table 2.

Table 2.
Preparation of Acetaldehyde standards

Well	2 mM Acetaldehyde Standard	Purified Water	Acetaldehyde (mM)
1	100 µL	-	2.0
2	60 µL	40 µL	1.2
3	30 µL	70 µL	0.6
4	-	100 µL	0

5. Mix well and transfer 20 µL of each Standard into separate wells.

Measurement

1. Add 80 µL of Working Reagent to each Standard and Sample well.
2. Add 80 µL of Blank Working Reagent to each Sample Blank well.
3. Mix well.
4. Incubate the plate for 30 minutes at room temperature.
5. Measure the optical density (OD) at 565 nm (OD_{565}).

Results

1. Subtract the 0 Standard OD_{565} reading from all readings.
2. Plot the corrected OD_{565} Standard readings against standard concentrations and determine the slope.
3. If the Sample OD_{565} value is higher than the OD_{565} for the corrected 2 mM Acetaldehyde Standard, dilute the sample in purified water and repeat the assay.
4. Calculate the acetaldehyde concentration of Sample.

Acetaldehyde (mM) =

$$\frac{OD_S - OD_{SB}}{Slope\ (mM^{-1})} \times DF$$

where:

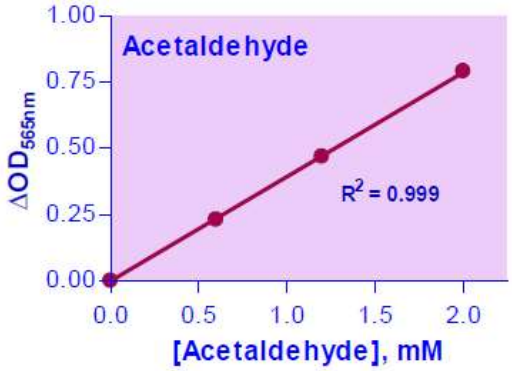
OD_S = OD_{565} reading of Sample

OD_{SB} = OD_{565} reading of Sample Blank

DF = Sample Dilution factor (DF = 1 for undiluted Samples)

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

Figure 1.
Typical Acetaldehyde Standard Curve



References

1. Lachenmeier, D.W., et. al., Carcinogenicity of acetaldehyde in alcoholic beverages: risk assessment outside ethanol metabolism. *Addiction,* **104(4)**:533-50 (2009).
2. Salaspuro, M., Acetaldehyde and gastric cancer. *J. Dig. Dis.*, **12(2)**: 51-9 (2011).
3. Seitz, H.K., et. al., Acetaldehyde as an underestimated risk factor for cancer development: role of genetics in ethanol metabolism. *Genes Nutri.*, **5(2)**: 121-28 (2010).

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