EnzyChrom™ Acetaldehyde Assay Kit (EACT-100)

Quantitative Colorimetric Acetaldehyde Determination at 565nm

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." Build up 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.

BioAssay Systems' 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)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Enzyme A:**	120 µL
NAD/MTT Solution:	1 mL	**Enzyme B:**	120 µL
3 M Standard:	100 µL		

Storage conditions. The kit is shipped on ice. Store components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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 on ice.

Important: only bring 3M Standard to room temperature for time needed to prepare standards. Return to -20°C within 30 minutes of thawing.

Reaction Preparation:

1. 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").
2. Prepare sufficient Working Reagent (WR) for the four 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*).
3. *Standards*. Make standards fresh, immediately before assay. Prepare 500 µL 30 mM Acetaldehyde by mixing 5 µL of the 3 M Standard and 495 µL distilled water. Prepare 300 µL of 2 mM Premix by mixing 20 µL 30 mM Acetaldehyde with 280 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table. Assay diluted standards within 10 minutes of preparation.

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

4. Transfer 20 µL standards into separate wells.
5. 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.
6. Read optical density at 565 nm (520-600 nm).

CALCULATION

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

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

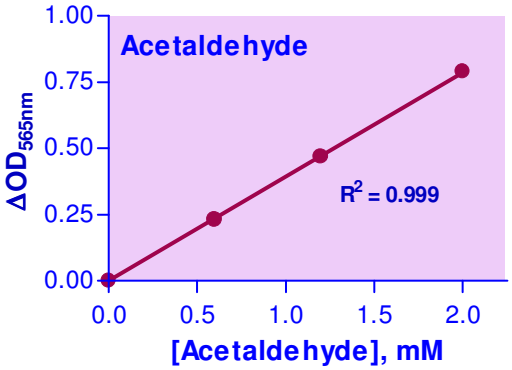
where OD_S and OD_{SB} are optical density readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.

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.

MATERIALS REQUIRED, BUT NOT PROVIDED

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



Standard Curve in 96-well plate assay in water.

LITERATURE

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