EnzyFluo™ Acetaldehyde Kit (EFAC-100)

Quantitative Fluorimetric Acetaldehyde Determination

DESCRIPTION

ACETALDEHYDE (CH_3CHO) 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." 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' fluorimetric 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 (50 µL sample): 0.5 to 60 µM

Convenient. The procedure involves adding a single working reagent, and reading the fluorescence after 30 minutes. Room temperature assay. No 37°C heater is needed.

High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to process 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).

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Enzyme A:**	120 µL
NAD Solution:	1 mL	**Enzyme B:**	120 µL
Probe:	750 µL	**3 M Standard:**	100 µL

Storage conditions. 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

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 solution, and Probe 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 50 µL of each sample **in duplicate** into separate wells (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: 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: 41 µL Assay Buffer, 8 µL NAD Solution, 5 µL Probe, and 1 µL Enzyme B. (*i.e. no Enzyme A*).
3. *Standards*. Make standards fresh, immediately before assay. Prepare 1 mL 15 mM Acetaldehyde by mixing 5 µL of the 3 M Standard and 995 µL distilled water. Prepare 1 mL of 60 µM Premix by mixing 4 µL 15 mM Acetaldehyde with 996 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	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

4. Transfer 50 µL standards into separate wells of a black, flat-bottom 96-well plate.
5. 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.
6. Read fluorescence at $\lambda_{ex/em}$ = 530/585 nm.

CALCULATION

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,

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

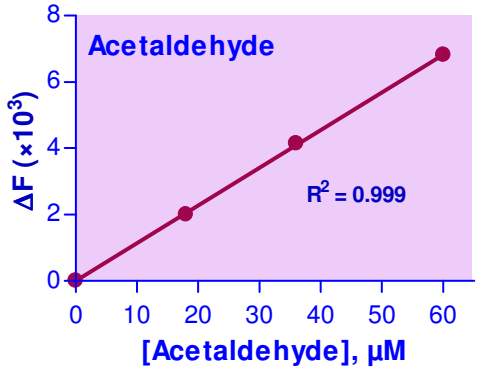
F_S and F_{SB} are fluorescence readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.

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.

MATERIALS REQUIRED, BUT NOT PROVIDED

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.



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, MI. (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.

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