

## **Technical Manual**

## **Acetaldehyde Assay Kit (Colorimetric)**

**Catalogue Code: BA0071**

**Pack Size: 100 assays**

**Research Use Only**

## DESCRIPTION

**ACETALDEHYDE** ( $CH_3CHO$ ) is one of the most widely occurring aldehydes in nature and is commonly used in industry. Acetaldehyde, a metabolic by-product 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.

The Assay Genie Acetaldehyde Assay Kit (Colorimetric) 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  $\mu$ L sample): 2  $\mu$ M to 2mM.

**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  $\mu$ L

**NAD/MTT Solution:** 1 mL      **Enzyme B:** 120  $\mu$ L

**Standard:** 100  $\mu$ L

**Storage conditions.** The kit is shipped on ice. Store components at -20°C upon receiving. Standard may be stored at 20°C or 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/MTT solution to room temperature. Briefly centrifuge tubes before use. Keep Enzymes and Standard on ice.

### Reaction Preparation:

1. Transfer 20  $\mu\text{L}$  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  $\mu\text{L}$  Assay Buffer, 8  $\mu\text{L}$  NAD/MTT, 1  $\mu\text{L}$  Enzyme A, and 1  $\mu\text{L}$  Enzyme B. Prepare sufficient Blank Working Reagent (BWR) for the "Sample Blank" wells by mixing, for each well: 75  $\mu\text{L}$  Assay Buffer, 8  $\mu\text{L}$  NAD/MTT, and 1  $\mu\text{L}$  Enzyme B. (i.e. no Enzyme A).
3. Standards. Make standards fresh, immediately before assay. Prepare 500  $\mu\text{L}$  30 mM Acetaldehyde by mixing 5  $\mu\text{L}$  of the 3 M Standard and 495  $\mu\text{L}$  distilled water. Prepare 300  $\mu\text{L}$  of 2 mM Premix by mixing 20  $\mu\text{L}$  30 mM Acetaldehyde with 280  $\mu\text{L}$  distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table. Assay diluted standards within 10 minutes of preparation.

No	2mM Premix + H <sub>2</sub> O	Acetaldehyde (mM)
1	100 $\mu\text{L}$ + 0 $\mu\text{L}$	2.0
2	60 $\mu\text{L}$ + 40 $\mu\text{L}$	1.2
3	30 $\mu\text{L}$ + 70 $\mu\text{L}$	0.6
4	0 $\mu\text{L}$ + 100 $\mu\text{L}$	0

4. Transfer 20  $\mu\text{L}$  standards into separate wells.
5. Add 80  $\mu\text{L}$  WR to the Standards and the "Sample" wells. Add 80  $\mu\text{L}$  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

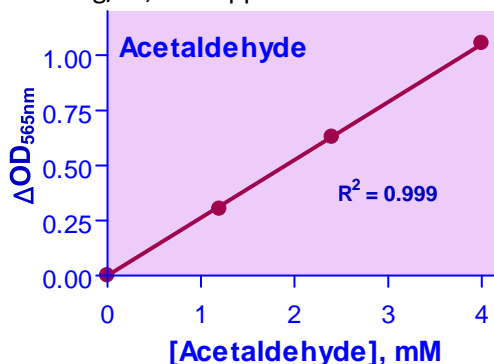
Subtract the blank value (#4) from the standard values and plot the  $\Delta\text{OD}$  against standard concentrations. Determine the slope and calculate the acetaldehyde concentration of Sample,

$$[\text{Acetaldehyde}] = \frac{\text{OD}_S - \text{OD}_{SB}}{\text{Slope (mM}^{-1})} \times n \text{ (mM)}$$

where  $\text{OD}_S$  and  $\text{OD}_{SB}$  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.



Standard Curve in 96-well plate assay in water.

#### **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.

#### **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.

### **Contact Details**

**Dublin, Ireland**

**Email:** [info@assaygenie.com](mailto:info@assaygenie.com)

**Web:** [www.assaygenie.com](http://www.assaygenie.com)

**Technical Support:** [Techsupport@assaygenie.com](mailto:Techsupport@assaygenie.com)

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