Acetaldehyde Assay Kit (Colorimetric)

Acetaldehyde Assay Kit (Colorimetric) is a detection kit for the quantification of Acetaldehyde in serum, plasma, urine, tissue, cell culture supernatants, food and beverage.

Catalog number: ARG82147

Package: 100 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Acetaldehyde (systematic name ethanal) is an organic chemical compound with the formula CH3CHO, sometimes abbreviated by chemists as MeCHO (Me = methyl). It is one of the most important aldehydes, occurring widely in nature and being produced on a large scale in industry. Acetaldehyde occurs naturally in coffee, bread, and ripe fruit, and is produced by plants. It is also produced by the partial oxidation of ethanol by the liver enzyme alcohol dehydrogenase and is a contributing cause of hangover after alcohol consumption. Pathways of exposure include air, water, land, or groundwater, as well as drink and smoke. Consumption of disulfiram inhibits acetaldehyde dehydrogenase, the enzyme responsible for the metabolism of acetaldehyde, thereby causing it to build up in the body.

The International Agency for Research on Cancer (IARC) has listed acetaldehyde as a Group 1 carcinogen. Acetaldehyde is "one of the most frequently found air toxins with cancer risk greater than one in a million".

In the liver, the enzyme alcohol dehydrogenase oxidizes ethanol into acetaldehyde, which is then further oxidized into harmless acetic acid by acetaldehyde dehydrogenase. These two oxidation reactions are coupled with the reduction of NAD^+ to NADH. [Wikipedia: Acetaldehyde]

PRINCIPLE OF THE ASSAY

This Acetaldehyde Assay Kit is simple and could be used to quantify Acetaldehyde from serum, plasma, urine, tissue, cell culture supernatants, food and beverage. In this Acetaldehyde Assay Kit, Acetaldehyde oxidized into harmless acetic acid by acetaldehyde dehydrogenase, as it converts NAD+ to NADH. The produced NADH could be used to reduce a tetrazolium salts, MTT, through enzyme reaction. The reduced form MTT is purple color and it can be detected by absorbance at 565 nm. The intensity of the purple color formed is directly proportional to the enzyme.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Assay Buffer	10 ml (ready to use)	-20°C
NAD/MTT Solution	1 ml (ready to use)	-20°C
Enzyme A	120 µl (ready to use)	-20°C
Enzyme B	120 µl (ready to use)	-20°C
Standard (3M Acetaldehyde)	100 µl (ready to use)	-20°C

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life of six months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 565 nm
- Clear, flat bottomed 96-well microplate
- Pipettes and pipette tips
- Multi-channel pipettes
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at -20°C at all times.
- Assays can be executed at room temperature.
- All components should be equilibrated to room temperature and briefly centrifuge all reagent before opening.
- Mix well all reagent and briefly spin down the reagents before use.
- This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated. All samples should be clear and free of any turbidity or particles. Liquid samples (e.g. non-hemolyzed serum, plasma, urine) can be assayed directly.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Collect serum and assay immediately or aliquot and store samples at ≤ -80°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using Citrate or heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -80°C. Avoid repeated freeze-thaw cycles.

Samples should be clear and free of particles or precipitates. Avoid using haemolytic, icteric or lipaemic samples.

Tissue lysate: Rinse tissue in ice-cold phosphate buffered saline (pH 7.4) to remove blood prior to dissection. Start with 20-100 mg tissue, add 200-1000 µL ice-cold PBS. Lysis can be achieved by homogenization (10-20 passes in a Dounce homogenizer on ice) or by sonication (preferably performed in an ice-water bath). The degree of tissue lysis can be checked under a microscope. Centrifuge homogenate at 14,000 g for 10 min. Transfer the clear supernatant into a clean tube. It is prudent to run a pilot test of the sample at different dilutions. Choose a dilution with the readings in the detection range of the

standard curve for further assays. Most samples can be stored at -80°C if not assayed immediately. Assay samples immediately or aliquot and store samples at ≤ -80°C. Avoid repeated freeze-thaw cycles.

Cell lysate: Wash cells with ice-cold phosphate buffered saline (pH 7.4) once. Suspend about 2 × 10^6 harvested cells in 400 µL PBS on ice. Lysis can be achieved by homogenization (10-20 passes in a Dounce homogenizer on ice) or by sonication (preferably performed in an ice-water bath). The degree of cell lysis can be checked under a microscope. Centrifuge homogenate at 14,000 g for 10 min. Transfer the clear supernatant into a clean tube. It is prudent to run a pilot test of the sample at different dilutions. Choose a dilution with the readings in the linear range of the standard curve for further assays. Most samples can be stored at -80°C if not assayed immediately. Assay samples immediately or aliquot and store samples at ≤ -80°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Assay Buffer, NAD/MTT Solution, Enzyme A, Enzyme B:** All reagents are ready to use, mix it well by vigorous shaking and centrifuge reagent tubes before use. Equilibrate Assay Buffer and NAD/MTT solution to room temperature prior to use. Keep Enzymes on ice before use.
- **Sample**: For unknown samples or if samples might contain Acetaldehyde higher than highest linear range of 2mM, the samples are suggested to perform several dilutions with distilled water to ensure that Acetaldehyde is within the linear range of 2µM to 2mM. For the calculation of the concentrations this dilution factor "N" has to be taken into account. The sample must be well mixed with the distilled water before assay.

  (It is recommended to do pre-test to determine the suitable dilution factor).
- **Standard:** only bring 3M Standard to room temperature for time needed to prepare standards. Return to -20°C within 30 minutes of thawing. Prepare standards immediately before assay, the diluted standards should be used within 10 minutes of preparation.

  Add 5 µl of the 3 M Standard stock into 495 µl of distilled water to yield a standard concentration of 30 mM Acetaldehyde standard. Prepare 300 µl of 2 mM S1 standard by mixing 20 µl of 30 mM Acetaldehyde standard with 280 µL distilled water.

  Dilute the 2 mM Acetaldehyde Standard solutions with distilled water to 1.2 mM and 0.6 mM as following table, and use distilled water serves as zero standard (0 mM).

Standard No.	Standard Conc. mM (mmol/L)	Distilled water (µl)	Standard (µl)
S1	2	280	20 of 30 mM standard
S2	1.2	40	60 of S1
S3	0.6	70	30 of S1
S0	0	100	0

- **Working Reagent (for standard S0-S3 and assayed samples):**

  For each reaction combine the following (*Prepare before use)*:

  75 µl of Assay Buffer

  8 µl of NAD/MTT

  1 µL of Enzyme A

  1 µL of Enzyme B

  Mix well, transfer 80 µl of Working Reagent to each assayed sample and standard wells.

- **Sample Blank Working Reagent (for sample blank):**

  For each reaction combine the following (*Prepare before use)*:

  75 µl of Assay Buffer

  8 µl of NAD/MTT

  1 µL of Enzyme B

  Mix well, transfer 80 µl of Sample Blank Working Reagent to each sample blank well. **(NO Enzyme A)**

ASSAY PROCEDURE

All components should be briefly mix and centrifuge reagent tubes before use. Standards and samples should be assayed in at least duplicates.

1. Add **20 µl** of **each sample** in two separate wells in a clear, flat bottomed 96 well plate. One is for assayed sample the other one well is for sample blank.
2. Prepare Working Reagent and Sample Blank Working Reagent as the REAGENT PREPARATION section as above.
3. Prepare standard as the REAGENT PREPARATION section as above. Add **20 µl** of **each standard (S0-S3)** to separate wells in the 96 well plate.
4. Add **80 µl** of **Working Reagent** into each **assayed sample** and **standard S0-S3 well**.
5. Add **80 µl** of **Sample Blank Working Reagent** into each **sample blank well**.
6. Tap the plate to mix it briefly and thoroughly immediately. Incubate at **room temperature for 30 minutes** in dark.
7. Read O.D. with a microplate reader at **565 nm (520-600 nm)** immediately.

Summary of Assay Procedure

	Assayed sample	Sample blank	Standard S0-S3
Sample	20 µl	20 µl	-
Standards	-	-	20 µl
Working Reagent for Standards and Samples	80 µl	-	80 µl
Sample Blank Working Reagent	-	80 µl	-
Mix well and incubate for 30 min at RT in dark.			
Read O.D. with a microplate reader at 565 nm (520-600 nm) immediately			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.
2. Subtract the blank value (S0) from the standard values and plot the △OD against standard concentrations. Determine the slope by △OD values of standards.
3. Acetaldehyde can then be calculated as follows:

   Acetaldehyde (mM)=

   $N \times [(OD_{Assayed\ Sample} - OD_{Sample\ Blank}) / Slope\ (mM^{-1})]$

   Note:

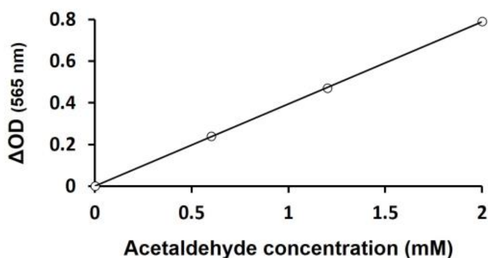
   $OD_{Assayed\ Sample} = OD_{565nm}$ of Assayed sample read at 30 min

   $OD_{Sample\ Blank} = OD_{565nm}$ of Sample blank read at 30 min

   N is the dilution factor (if sample has been diluted)
4. Conversions: 1 mM acetaldehyde equals 4.4 mg/dL, or 44 ppm.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Acetaldehyde Assay Kit was 2 µM. The Linear detection range ranged from 2 µM – 2 mM (for 20 µl of samples).

Limitation

The assay mechanism uses the enzyme aldehyde dehydrogenase to quantify acetaldehyde in the sample. The enzyme will also oxidize other aldehydes in the sample (i.e. propionaldehyde). Therefore, the assay will not distinguish between acetaldehyde and other aldehydes present in the sample. Consideration should be taken if high levels of other aldehydes are expected in the sample being tested.