

Acetaldehyde Assay Kit

Catalog No.: abx294450

Size: 96 tests

Detection Range: 2.8 $\mu\text{mol/L}$ – 56 $\mu\text{mol/L}$

Sensitivity: 2.8 $\mu\text{mol/L}$

Storage: Store all components at -20°C in the dark.

Application: For the detection and quantification of Acetaldehyde concentration in serum, plasma and wine.

Principle of the Assay

Abbexa's Acetaldehyde Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Acetaldehyde concentration. The Enzyme Reagent in the kit catalyzes the reaction between the Chromogenic Reagent and acetaldehyde, forming an orange-red product with a maximum absorbance of 450 nm. The intensity of the color is proportional to the Acetaldehyde concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer solution: 14 ml
3. Substrate: 6 vials
4. Enzyme Reagent: 2 vials
5. Chromogenic Reagent: 13 ml
6. Standard (0.112 mmol/L): 0.45 ml
7. Plate Sealer: 2

Materials required but not provided

1. Microplate reader (450 nm)
2. Double-distilled water
3. Pipette and pipette tips
4. Centrifuge and centrifuge tubes
5. Vortex mixer
6. Incubator

Instructions for Use

Version: 1.0.1

Revision date: 1-Oct-26



Protocol

A. Preparation of samples and reagents

1. Reagents

- **Substrate Working Solution:** Reconstitute 1 vial of Substrate with 1 ml of Buffer Solution. Mix thoroughly, until fully dissolved. Keep in the dark and on ice during use. Unused Substrate Working Solution can be stored at -20°C for up to 5 days.
- **Enzyme Working Solution:** Reconstitute 1 vial of Enzyme Reagent with 1.2 ml of Buffer Solution. Mix thoroughly, until fully dissolved. Keep in the dark and on ice during use. Unused Enzyme Working Solution can be stored at -20°C for up to 15 days.
- **56 µmol/L Standard:** Dilute the stock Standard (56 µmol/L) 2-fold with double-distilled water. For example, add 250 µl of Standard (56 µmol/L) to 250 µl of double-distilled water and mix thoroughly. Store the prepared standard at 2-8°C for up to 30 days. As the standard is volatile, minimize exposure to air and reseal promptly after use.
- **Standards:** Label 7 tubes with 11.2 µmol/L, 16.8 µmol/L, 22.4 µmol/L, 28 µmol/L, 33.6 µmol/L, 39.2 µmol/L, and 56 µmol/L. Prepare these dilutions according to the volumes in the following table:

Standard Dilution (µmol/L)	11.2	16.8	22.4	28	33.6	39.2	56
56 µmol/L Standard (µl)	20	30	40	50	60	70	100
Double-distilled water (µl)	80	70	60	50	40	30	0

For the blank, use pure double-distilled water. The volume of each standard dilution will be 100 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- Always prepare a fresh set of standards and discard any unused working standard dilutions after use.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum, plasma and wine (liquid):** Samples can be tested directly.

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It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with double-distilled water, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human Serum	1
Aged Vinegar	5 – 10
White Spirits	4 – 10
Green Plum Wine	4 – 10

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

B. Assay Procedure

- Mark positions on the 96-well microplate for each standard, blank, and sample. *Each sample requires a corresponding control. It is strongly recommended to test all samples in duplicate.*
- Add 20 µl of each standard dilution to the standard wells.
- Add 20 µl of double-distilled water to the blank wells.
- Add 20 µl of sample to the sample wells.
- Add 50 µl of Substrate Working Solution to all wells.
- Add 20 µl of Enzyme Working Solution to all wells.
- Add 100 µl of Chromogenic Reagent to all wells.
- Mix the well contents by tapping the plate, or shaking with a microplate shaker, for at least 5 seconds.
- Cover with a plate sealer, then incubate at 37°C for 40 minutes.
- Unseal the plate, then measure the OD of each well with a microplate reader at 450 nm.

C. Calculation of Results

Plot the standard curve, using the mean OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$.

Based on this curve, the concentration of Acetaldehyde in each sample well can be derived using the following formula:

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Liquid samples:

$$\text{Acetaldehyde } (\mu\text{mol/L}) = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} - b)}{a} \times F$$

where:

OD _{Sample}	OD value of the sample.
OD _{Control}	OD value of the corresponding control
a	Gradient of the standard curve ($y = ax + b$)
b	Y-intercept of the standard curve ($y = ax + b$)
F	Dilution factor of the sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.