（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K769-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader (445-455 nm)

Detection range：2.80-56.00 μmol/L

Elabscience® Acetaldehyde Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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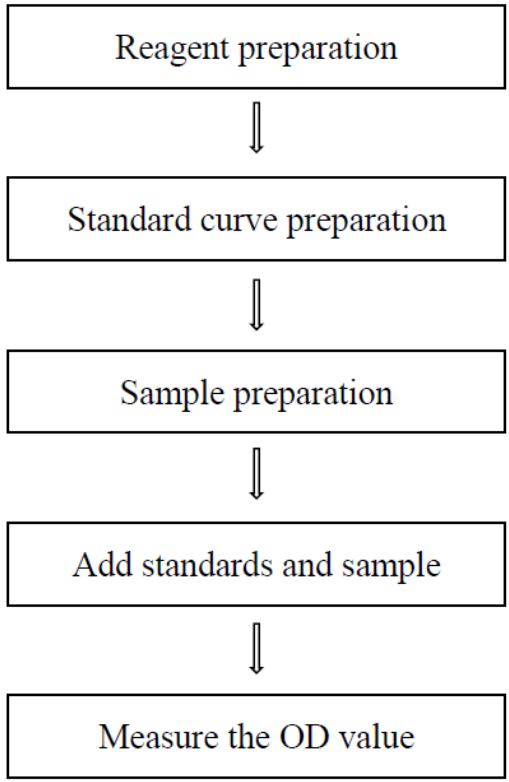
Assay summary



Intended use

This kit can be used to measure acetaldehyde content in serum, plasma and wine samples.

Detection principle

Acetaldehyde is the main aldehyde compound in fermented food such as beer and wine, and has pungent odor. At present, acetaldehyde can be used as a food additive in yogurt, juice, candy, and alcoholic beverages, and it is generally believed that acetaldehyde ingestion from normal drinking in daily life will not cause cancer. However, more and more recent studies have found that acetaldehyde produced in the human body through the pathway of ethanol metabolism will increase the probability of human cancer.

The detection principle of this kit is: The enzyme catalyzes acetaldehyde to product an orange-red substance with the chromogenic agent. The maximum absorption of the substance is at 450 nm, and the content of acetaldehyde in the sample can be calculated by measuring the OD value at 450 nm.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Buffer Solution	8 mL ×1 vial	14 mL ×1 vial	-20 ℃, 12 months, shading light
Reagent 2	Substrate	Powder ×3 vials	Powder ×6 vials	-20 ℃, 12 months, shading light
Reagent 3	Enzyme Reagent	Powder ×1 vial	Powder ×2 vials	-20 ℃, 12 months, shading light
Reagent 4	Chromogenic Agent	7 mL ×1 vial	13 mL ×1 vial	-20 ℃, 12 months, shading light
Reagent 5	0.112 mmol/L Standard Solution	0.45 mL ×1 vial	0.45 mL ×1 vial	-20 ℃, 12 months, shading light
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (445-455 nm, optimum wavelength: 450 nm)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of substrate working solution:

Dissolve one vial of substrate with 1 mL of buffer solution, mix well to dissolve. Keep it on ice protected from light during use. Store at -20 ℃ for 5 days protected from light.

③ The preparation of enzyme working solution:

Dissolve one vial of enzyme reagent with 1.2 mL of buffer solution, mix well to dissolve. Keep it on ice protected from light during use. Aliquoted storage at -20 ℃ for 15 days protected from light.

④ The preparation of 56 μmol/L standard solution:

Before testing, please prepare sufficient 56 μmol/L standard solution. For example, prepare 500 μL of 56 μmol/L standard solution (mix well 250 μL of 0.112 mmol/L standard solution and 250 μL of double distilled water). The 56 μmol/L standard solution can be sealed and stored at 2-8 ℃ for a month. (The standard solution is volatile, please take it quickly after opening and seal it).

⑤ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 56 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 11.2, 16.8, 22.4, 28.0, 33.6, 39.2, 56.0 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**11.2**	**16.8**	**22.4**	**28.0**	**33.6**	**39.2**	**56.0**
56 μmol/L Standard (μL)	0	20	30	40	50	60	70	100
Double distilled water (μL)	100	80	70	60	50	40	30	0

Sample preparation

① Sample preparation

Serum (plasma) and wine (liquid) samples: detect directly.

② Dilution of sample

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: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 20 μL of standard with different concentrations to the wells.

Sample well: Add 20 μL of sample to the wells.

② Add 50 μL of substrate working solution to each well.

③ Add 20 μL of enzyme working solution to each well.

④ Add 100 μL of chromogenic agent to each well.

⑤ Mix fully with microplate reader for 5 s. Incubate at 37 ℃ for 40 min and measure the OD value at 450 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Liquid sample:

$$\text{Acetaldehyde content (μmol/L)} = (\Delta A_{450} - b) \div a \times f$$

[Note]

ΔA_{450}: $\Delta A_{450} = OD_{sample} - OD_{blank}$.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	15.00	25.00	40.00
%CV	2.0	2.1	2.0

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	15.00	25.00	40.00
%CV	2.7	4.0	3.2

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 97.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	15	25	40
Observed Conc. (μmol/L)	14.3	25.0	39.2
Recovery rate (%)	95	100	98

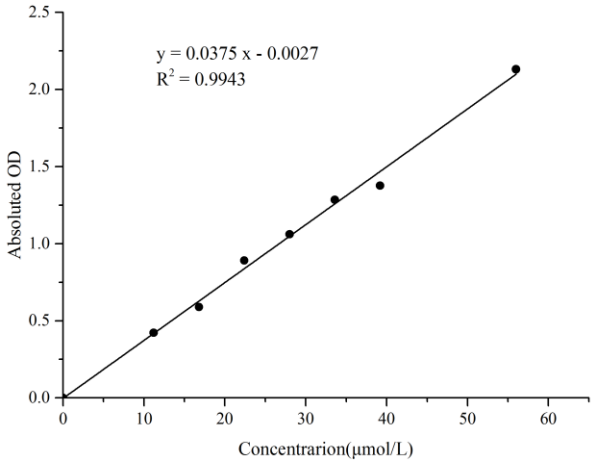
Sensitivity

The analytical sensitivity of the assay is 2.80 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**11.20**	**16.8**	**22.4**	**28.0**	**33.6**	**39.2**	**56.0**
OD Value	0.102	0.524	0.701	0.989	1.164	1.373	1.492	2.249
	0.105	0.527	0.684	1.001	1.165	1.403	1.468	2.221
Average OD	0.104	0.526	0.693	0.995	1.165	1.388	1.480	2.235
Absoluted OD	0	0.422	0.589	0.892	1.061	1.285	1.377	2.132



Appendix II Example Analysis

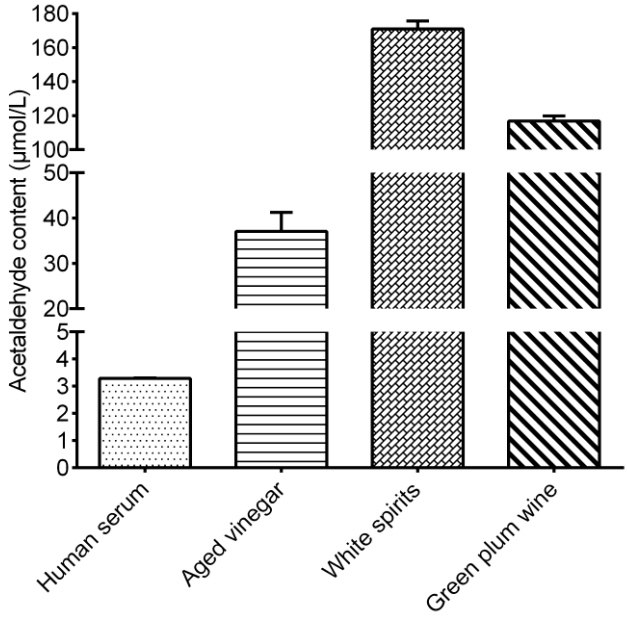
Example analysis:

Take 20 μL of white spirits which dilute for 4 times, and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0375 x - 0.0027, The OD value of the blank well is 0.104, the OD value of the sample well is 1.704, and the calculation result is:

$$\text{Acetaldehyde content (μmol/L)} = (1.600 + 0.0027) \div 0.0375 \times 4 = 170.95 \text{ μmol/L}$$

Detect human serum, aged vinegar (dilute for 7 times), white spirits (dilute for 4 times) and green plum wine (dilute for 4 times), according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.