Ethanol Assay Kit

Catalog No.: abx298912

Size: 96 tests

Detection Range: 0.27 µmol/ml – 10 µmol/ml

Sensitivity: 0.27 µmol/ml

Storage: Store all components at -20°C for up to 12 months. Store the Enzyme Reagent, Chromogenic Reagent, and the Substrate in the dark.

Application: For detection and quantification of Ethanol content in serum, plasma, and wine.

Introduction

Ethanol is commonly produced in nature as the final reaction product of anaerobic respiration in many plants, fungi, and bacteria. As well as being an essential solvent and intermediate in the chemical and manufacturing industries, ethanol is also widely used in the production of alcoholic beverages. This process is known as fermentation.

Abbexa's Ethanol Assay Kit is a quick, convenient, and sensitive method for measuring Ethanol content. The enzyme ethanol dehydrogenase can oxidize Ethanol to acetaldehyde, reducing NAD^+ to NADH in the process. NADH facilitates the production of a yellow compound, with an absorbance maximum at 450 nm. The intensity of the color is proportional to the Ethanol concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Diluent A: 20 ml
3. Diluent B: 14 ml
4. Enzyme Reagent: 2 vials
5. Chromogenic Reagent: 2 × 1.5 ml
6. Substrate: 2 vials
7. Standard (10 µmol/ml): 2 × 1.8 ml
8. Plate sealer: 2

Materials required but not provided

1. Microplate reader (450 nm)
2. Distilled water
3. Pipette and pipette tips
4. 1.5 ml microcentrifuge tubes
5. Vortex mixer
6. Incubator

Protocol

A. Preparation of samples and reagents

1. 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** and **Plasma**: Serum and plasma samples can be tested directly.
- **Wine**: Alcoholic beverages can be tested directly.

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 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
Beers (2.8% alcohol)	60 – 100
White wines (12% alcohol)	250 – 300

Note:

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

2. Reagents

- **Enzyme Reagent Working Solution**: Dissolve the powder in 180 µl of Distilled water and mix fully. Allow to stand for 30 minutes before use. The prepared solution can be stored in the dark at 4°C for up to 2 days.
- **Substrate Working Solution**: Dissolve the powder in 170 µl of Distilled water and mix fully. The prepared solution can be stored in the dark at -20°C for up to 2 days.
- **Reaction Working Solution**: Mix Diluent A, Diluent B, Enzyme Reagent Working Solution, Substrate Working Solution, and the Chromogenic Reagent in a ratio of 80 : 37 : 1 : 2 : 8. Mix fully, and keep in the dark. The Reaction Working Solution must be used within 30 minutes. It is recommended that this solution is only prepared once the samples and standards have been added to the plate wells (**Assay Procedure - Step B.3**)
- **Standards**: Label 7 tubes with 10 µmol/ml, 9 µmol/ml, 8 µmol/ml, 6 µmol/ml, 4 µmol/ml, 3 µmol/ml, and 2 µmol/ml. Add 200 µl, 180 µl, 160 µl, 120 µl, 80 µl, 60 µl, and 40 µl of Standard Solution (10 µmol/ml) to the 10 µmol/ml, 9 µmol/ml, 8 µmol/ml, 6 µmol/ml, 4 µmol/ml, 3 µmol/ml, and 2 µmol/ml tubes respectively, followed by 0 µl, 20 µl, 40 µl, 80 µl, 120 µl, 140 µl, and 160 µl of Distilled water, to prepare the Standard Dilutions with concentrations 10 µmol/ml, 9 µmol/ml, 8 µmol/ml, 6 µmol/ml, 4 µmol/ml, 3 µmol/ml, and 2 µmol/ml. These volumes are summarized in the following table:

Standard Dilution (µmol/ml)	10	9	8	6	4	3	2
10 µmol/ml Standard (µl)	200	180	160	120	80	60	40
Distilled water (µl)	0	20	40	80	120	140	160

For the blank, or 0 mg/ml standard, use pure Distilled water. The volume of each standard will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

1. Mark the positions of each standard, sample, and blank. It is strongly recommended to test all wells in duplicate.
2. Add 40 µl of each Standard Dilution to their corresponding wells.
3. Add 40 µl of each sample to the sample wells. Pipette samples gently up and down to mix before adding to wells. Avoid foaming or bubbles.
4. Add 160 µl of Reaction Working Solution to all wells.
5. Mix fully for at least 3 seconds.
6. Within 2 minutes of adding the Reaction Working Solution, measure and record the OD of each well with a microplate reader at 450 nm. Mark these values as $OD_{Initial}$.
7. Incubate in the dark at 37°C for 10 minutes.
8. Measure and record the OD of each well with a microplate reader at 450 nm. Mark these values as OD_{Final}.

C. Calculation of Results

Plot the standard curve, using the difference in OD of the standard dilutions, adjusted for the difference in the blank ($\mathrm{OD_{Final}} - \mathrm{OD_{Initial}} - \mathrm{OD_{Blank}}$), 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 Ethanol in each sample well can be derived with the formula:

1. Liquid Samples:

$$\mathbf{Ethanol\ (\mu mol/ml) = F \times \frac{(OD_{Difference} - OD_{Blank} - b)}{a}}$$

where:

$\mathrm{OD_{Difference}}$	Difference in OD of the sample well between the initial reading and reading after 10 minutes ($\mathrm{OD_{Final}} - \mathrm{OD_{Initial}}$)
$\mathrm{OD_{Blank}}$	Difference in OD of the blank well between the initial reading and reading after 10 minutes ($\mathrm{OD_{Final}} - \mathrm{OD_{Initial}}$)
a	Gradient of the standard curve ($y = ax + b$)
b	Y-intercept of the standard curve ($y = ax + b$)
F	The dilution factor of sample