# Amplite® Ethanol Quantitation Kit

Catalog number: 40001
Unit size: 200 Tests

| Component | Storage | Amount (Cat No. 40001) |
|---|---|---|
| Component A: Amplite® Ethanol Reagent (light sensitive) | Freeze (< -15 °C), Minimize light exposure | 1 vial |
| Component B: Assay Buffer | Freeze (< -15 °C) | 1 bottle (10 mL) |
| Component C: Ethanol Enzyme Mix (lyophilized) | Freeze (< -15 °C), Minimize light exposure | 1 vial |
| Component D: DMSO | Freeze (< -15 °C) | 1 vial (200 µL) |
| Component E: Ethanol Standard | Freeze (< -15 °C), Minimize light exposure | (100%, 0.5 mL) |

## OVERVIEW

Amplite® Ethanol Quantitation Kit is a fast, non-radioactive assay designed for the sensitive detection of ethanol in biological and industrial samples. Based on the enzymatic oxidation of ethanol by alcohol oxidase, which is then detected using Amplite® Red, a proprietary fluorogenic and chromogenic substrate.

The kit supports both fluorimetric and colorimetric detection modes, allowing flexible integration into diverse assay platforms. With a simple mix-and-read protocol, the assay is completed in under 30 minutes and is easily scalable for high-throughput applications. It is ideal for life science research, clinical evaluations, and ethanol quantitation in food and beverage testing.

## AT A GLANCE

### Protocol Summary

1. Prepare Ethanol standards or test samples (50 µL)
2. Add Ethanol working solution (50 µL)
3. Incubate at room temperature for 5 - 30 minutes
4. Monitor fluorescence intensity at Ex/Em = 540/590 nm (Cutoff = 570 nm)

### Important Note

Thaw all the kit components to room temperature before starting the experiment.

## KEY PARAMETERS

### Fluorescence microplate reader

| | |
|---|---|
| Cutoff | 570 nm |
| Emission | 590 nm |
| Excitation | 540 nm |
| Recommended plate | Solid black |

### Absorbance microplate reader

| | |
|---|---|
| Absorbance | 576 ± 5 nm |
| Recommended plate | Clear bottom |

## PREPARATION OF STOCK SOLUTIONS

*Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles*

### Amplite™ Ethanol Reagent stock solution (250X)

Add 40 µL of DMSO (Component D) into the vial of Amplite™ Ethanol Reagent (Component A) to make 250X Amplite™ Ethanol Reagent stock solution. The stock solution should be used promptly.

**Note:** The Amplite™ Ethanol Reagent is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. The final concentration of DTT or 2-mercaptoethanol in the reaction should be no higher than 10 µM. The Amplite™ Ethanol Reagent is also unstable at high pH (>8.5). Therefore, the reaction should be performed at pH 7 – 8. The provided assay buffer (pH 7.4) is recommended.

### Ethanol Enzyme Mix (100X)

Add 100 µL of Assay Buffer (Component B) into the vial of Ethanol Enzyme Mix (Component C) and mix well to make 100X Ethanol Enzyme Mix.

## PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/40001

### Ethanol standard

Prepare an Ethanol standard by diluting the appropriate amount of the 100% Ethanol Standard (Component E) into H2O to produce Ethanol concentration ranging from 0% to 0.1%. A 0% Ethanol control is included as blank control. The final Ethanol concentrations should be two folds lower (i.e., 0% to 0.05%).

## PREPARATION OF WORKING SOLUTION

Add 20 µL of 250X Amplite™ Ethanol Reagent Stock Solution and 50 µL of 100X Ethanol Enzyme Mix into 5 mL of Assay Buffer (Component B) to make Ethanol working solution.

## SAMPLE EXPERIMENTAL PROTOCOL

**Table 1.** Layout of Ethanol standards and test samples in a solid black 96-well microplate. ES= Ethanol Standards (ES1 - ES7, 0.0001% to 0.1%), BL=Blank Control, TS=Test Samples.

| BL | BL | TS | TS |
|---|---|---|---|
| ES1 | ES1 | ... | ... |
| ES2 | ES2 | ... | ... |
| ES3 | ES3 | | |
| ES4 | ES4 | | |
| ES5 | ES5 | | |
| ES6 | ES6 | | |
| ES7 | ES7 | | |

**Table 2.** Reagent composition for each well.

| Well | Volume | Reagent |
|---|---|---|
| ES1 - ES7 | 50 µL | Serial Dilutions (0.0001% to 0.1%) |
| BL | 50 µL | $H_2O$ |
| TS | 50 µL | test sample |

1. Prepare Ethanol standards (ES), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

   **Note:** High concentration of Ethanol (e.g. 0.05% final concentration) may cause reduced fluorescence signal due to the over oxidation of Amplite™ ethanol reagent (to a non-fluorescent product).

2. Add 50 µL of Ethanol working solution to each well of Ethanol standard, blank control, and test samples to make the total Ethanol assay volume of 100 µL/well. For a 384-well plate, add 25 µL of Ethanol working solution into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction at room temperature for 5 to 30 minutes, protected from light.
4. Monitor the fluorescence intensity with a fluorescence plate reader at Excitation = 530 - 570 nm, Emission = 590 - 600 nm (optimal Ex/Em = 540/590 nm), Cutoff = 570 nm.

   **Note:** The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576 ± 5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

## EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate EtOH samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

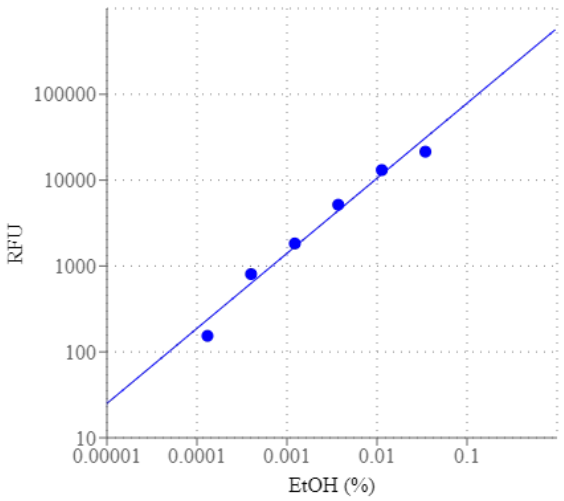


**Figure 1.** Ethanol dose response was measured with Amplite® Ethanol Quantitation Kit on a solid black 96-well plate using a Gemini fluorescence microplate reader (Molecular Devices).

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