

Amplite® Total Alcohol Quantification Assay Kit

Catalog number: 40003
Unit size: 100 tests

Component	Storage	Amount (Cat No. 40003)
Component A: Amplite® Total Alcohol Reagent (light sensitive)	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (10 mL)
Component C: Alcohol Enzyme Mix (lyophilized)	Freeze (< -15 °C), Minimize light exposure	1 vial
Component D: DMSO	Refrigerated (2-8 °C)	1 vial (200 µL)
Component E: Ethanol Standard	Freeze (< -15 °C), Minimize light exposure	(100%, 0.5 mL)

OVERVIEW

Amplite® Total Alcohol Quantification Assay Kit is a fast, non-radioactive assay designed for the sensitive detection of total alcohol content in biological samples. It uses a proprietary reaction detected with Amplite® Red, a fluorogenic and chromogenic substrate that allows versatile signal measurement.

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 total alcohol quantitation in food and beverage testing.

AT A GLANCE

Protocol Summary

1. Prepare Ethanol standards or test samples (100 µL)
2. Add Alcohol working solution (100 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™ Total Alcohol 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.

Alcohol Enzyme Mix (100X)

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

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
<https://www.aatbio.com/tools/serial-dilution/40003>

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 40 µL of 250X Amplite™ Total Alcohol Reagent Stock Solution and 100 µL of 100X Alcohol Enzyme Mix into 10 mL of Assay Buffer (Component B) to make Alcohol 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.

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Well	Volume	Reagent
ES1 - ES7	100 µL	Serial Dilutions (0.0001% to 0.1%)
BL	100 µL	H ₂ O
TS	100 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 100 µL.

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

2. Add 100 µL of alcohol working solution to each well of ethanol standard, blank control, and test samples to make the total assay volume of 200 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 4PL calculator which can be found at:

<https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator>

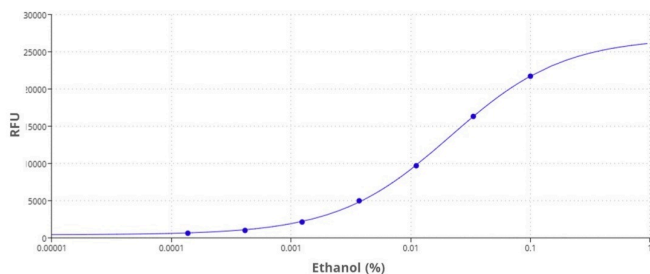


Figure 1. Ethanol content was measured with Amplite® Total Alcohol Quantitation Assay Kit (Cat# 40003) on a solid black 96-well plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER

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