Amplite™ Ethanol Quantitation Kit

Catalog number: 40001
Unit size: 200 Tests

Component	Storage	Amount
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

The ability to rapidly perform quantitative measurements of ethanol is highly desirable in life science research, clinical evaluations, and food and drug industries. This non-radioactive ethanol assay is based on the oxidation of ethanol by alcohol oxidase. The kit uses our Amplite™Red that makes the kit recordable in a dual mode, either fluorimetric or colorimetric readout. The kit provides all the essential components with an optimized assay protocol. The assay is robust, sensitive, and can be readily adapted for high throughput assays in a wide variety of applications that require the measurement of ethanol. The assay can be completed within 30 minutes.

AT A GLANCE

Protocol summary

1. Prepare Ethanol working solution (50 µL)
2. Add Ethanol standards or test samples (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 Thaw all the kit components to room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black
Instrument:	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.

1. 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.

2. 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 SOLUTION

Ethanol standard

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

Prepare an Ethanol standard by diluting the appropriate amount of the 100% Ethanol Standard (Component E) into H_2O 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. 0.5% final concentration) may cause reduced fluorescence signal due to the over oxidation of Amplite™ ethanol reagent (to a non-fluorescent product).

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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 base-line 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

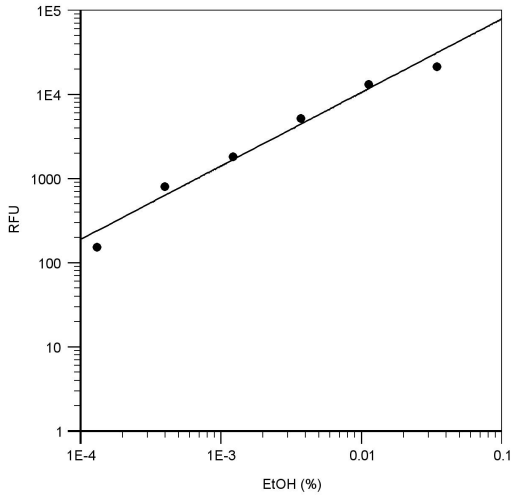

Figure 1. Ethanol dose response was measured with Amplite™ Ethanol Quantitation Kit on a solid black 96-well plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER