Amplite™ Ethanol Quantitation Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 40001 (200 assays)	Keep at -20 °C and protect from light	Fluorescence microplate readers

Introduction

Ethanol is a powerful psychoactive drug and one of the oldest recreational drugs. It is best known as the type of alcohol found in alcoholic beverages and thermometers. In common usage, it is often referred to simply as alcohol or spirits. Ethanol is a central nervous system depressant and has significant psychoactive effects in sublethal doses. A blood ethanol level of 0.5% or more is commonly fatal. Ethanol levels of even less than 0.1% can cause intoxication, with unconsciousness often occurring at 0.3 - 0.4%. The amount of ethanol in the body is typically quantified by blood alcohol content.

The ability to rapidly perform quantitative measurements of ethanol is highly desirable in life science research, clinical evaluations, food, and pharmaceutical industries. Our non-radioactive ethanol assay is based on the oxidation of ethanol by alcohol oxidase. The kit uses our Amplite™ Red reagent that makes the kit recordable in a dual mode, the fluorescent signal can be easily read by a fluorescence microplate reader at Ex/Em = ~540/590 nm, or its absorption can be readily read by an absorbance microplate reader at ~570 nm. The Amplite™ Fluorimetric Ethanol Quantitation Kit can be performed in a convenient 96-well or 384-well microtiter-plate format. The assay can be completed within 30 minutes and as little as 0.0003% ethanol can be detected.

Kit Key Features

Sensitive:	Detect as low as 0.0003% Ethanol in solution.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	**Amount**
Component A: Amplite™ Reagent (light sensitive)	1 vial
Component B: Assay Buffer	1 bottle (10 mL)
Component C: Ethanol Enzyme Mix (lyophilized)	1 vial
Component D: DMSO	1 vial (200 µL)
Component E: Ethanol Standard	0.5 mL

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare assay reaction mixture (50 µL) → Add Ethanol standards or test samples (50 µL) → Incubate at room temperature for 5 - 30 minutes→ Monitor fluorescence intensity at Ex/Em = 540/590 nm

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

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1. Prepare stock solutions:

1.1 250X Amplite™ Reagent stock solution: Add 40 µL of DMSO (Component D) into the vial of Amplite™ reagent (Component A). The stock solution should be used promptly. Any remaining solution should be aliquoted and refrozen at -20 °C.
Note 1: Avoid repeated freeze-thaw cycles.
Note 2: The Amplite™ 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™ 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.

1.2 100X Ethanol Enzyme Mix: Add 100 µL of Assay Buffer (Component B) into the vial of Ethanol Enzyme Mix (Component C), and mix well.
Note: The unused Ethanol enzyme mix solution should be divided into single use aliquots and stored at -20 °C.

2. Prepare assay reaction mixture:

Prepare assay reaction mixture according to the following tables, protected from light.

Table 1 Assay reaction mixture for one 96-well plate

Components	**Volume**
250X Amplite™ Reagent Stock Solution (from Step 1.1)	20 µL
100X Ethanol Enzyme Mix (from Step 1.2)	50 µL
Assay Buffer (Component B)	5 mL
Total volume	5.07 mL

Table 2 Layout of ethanol standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
ES1	ES1	….	….	….	….						
ES2	ES2										
ES3	ES3										
ES4	ES4										
ES5	ES5										
ES6	ES6										
ES7	ES7										

Note: ES= Ethanol Standards, BL=Blank Control, TS=Test Samples.

Table 3 Reagent composition for each well

Ethanol Standard	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	H_2O: 50 µL	50 µL

**Note 1: Add the serial dilutions of Ethanol standard from 0.0001% to 0.1% into each well from ES1 to ES7 in duplicate.*
Note 2: High concentration of Ethanol (e. g., 0.5%, final concentration) may cause reduced fluorescence signal due to the overoxidation of Amplite™ ethanol substrate (to a non-fluorescent product).

3. Run ethanol assay:

3.1 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%, each in a volume of 50 µL. A 0% ethanol control is included as blank control. The final ethanol concentrations should be two folds lower (i.e., 0 to 0.05%).

3.2 Add 50 µL of assay reaction mixture (from Step 2) into each well of ethanol standard, blank control, and test samples (see Step 2, Table 3) to make the total ethanol assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of assay reaction mixture *into each well.*

3.3 Incubate the reaction for 5 to 30 minutes at room temperature, protected from light.

3.4 Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em= 530-570 nm/590-600 nm (optimal Ex/Em = 540/590 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.

Data Analysis

The fluorescence in blank wells (with the H_2O only) is used as a control, and is subtracted from the values for those wells with ethanol reactions. The typical data are shown in Figure 1 (ethanol standard curve).
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

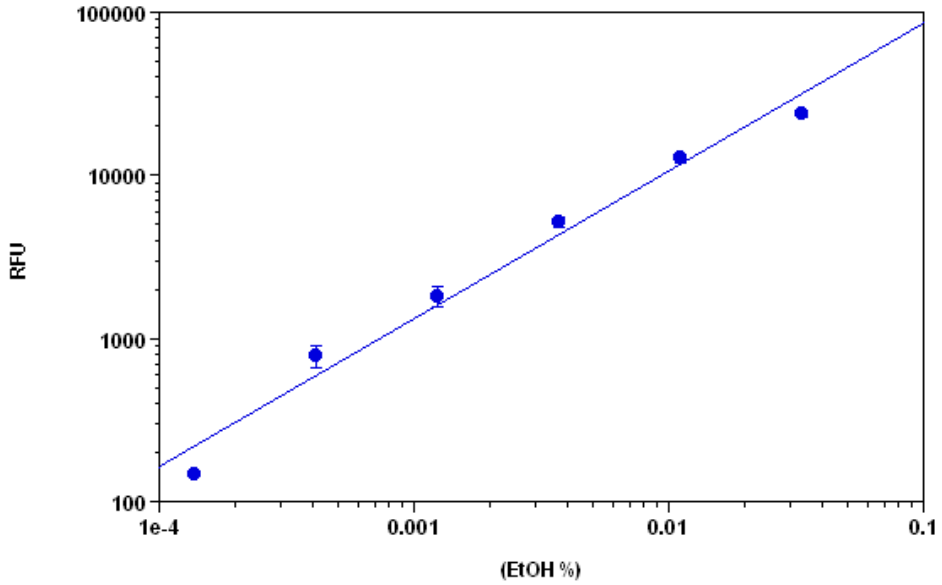

Figure 1. Ethanol dose response was measured with Amplite™ Fluorimetric Ethanol Quantitation Kit on a 96-well black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.0003% of Ethanol was detected with 15 minutes incubation time (n=3).

References

1. Peterson KP, Bowers C, Peterson CM. (1998) Prevalence of ethanol consumption may be higher in women than men in a university health service population as determined by a biochemical marker: whole blood-associated acetaldehyde above the 99th percentile for teetotalers. J Addict Dis, 17, 13.
2. Chen HM, Peterson CM. (1994) Quantifying ethanol by high performance liquid chromatography with precolumn enzymatic conversion and derivatization with fluorimetric detection. Alcohol, 11, 577.
3. Linares P, Ruz J, De Castro MD, Valcarcel M. (1987) Enzymatic determination of ethanol in saliva by flow injection analysis. J Pharm Biomed Anal, 5, 701.
4. Fernandez Gomez A, Ruz Polonio J, Luque de Castro MD, Valcarcel Cases M. (1985) Automatic enzymatic-fluorimetric determination of ethanol in blood by flow injection analysis. Clin Chim Acta, 148, 131.