Amplite™ Ethanol Quantitation Kit *Red Fluorescence*

Ordering Information:	Storage Conditions:
Product Number: #40001 (200 assays)	Keep in freezer and avoid light

Introduction

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™ reagent that maks the kit recordable in a dual mode, the fluorescent signal can be easily read by either fluorescence microplate reader with Ex/Em = 530 to 570 nm/590 to 600 nm (maximum Ex/Em = 540 nm/590 nm) or absorbance microplate reader at 576±5 nm.

The Amplite™ Ethanol Quantitation Kit can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. The assay can be completed within 30 minutes. With the Amplite™ Ethanol Quantitation Kit, we have detected as little as 0.0003% Ethanol.

Kit Key Features

Sensitive:	The kit detects as low as 0.0003% Ethanol in solution.
Continuous:	Easily adapted to automation with no separation required.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Component	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 1 plate)

Brief Summary

Prepare assay reaction mixture (50 µL) → Add Ethanol standards or test samples (50 µL) → Incubate at room temperature for 5-30 min → Read fluorescence at Ex 540 nm/Em 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 Amplite™ Reagent stock solution (250X): Add 40 µL of DMSO (Component D) into the vial of Amplite™ reagent substrate (Component A). The stock solution should be used promptly. Any remaining solution need 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. It is also unstable at high pH (>8.5). The reactions should be performed at pH 7–8. The provided assay buffer, pH 7.4, is recommended.

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

2. Prepare assay reaction mixture:

2.1 Prepare Assay reaction mixture according to the following table and kept from light:

Table 1. Assay reaction mixture for one 96-well plate (2X)

Components	**Volume**
Amplite™ Reagent stock solution (250X, from step 1.1)	20 uL
Ethanol enzyme mix: (100X, from step 1.2)	50 uL
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)	H2O (Component B): 50 µL	50 µL

**Note 1: Add the serially diluted Ethanole standards from 0.0001% to 0.1% into wells 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 of 0 to 0.1%, each in a volume of 50 µL. A 0% ethanol control is included as blank control. The final ethanol concentrations will be twofold lower (i.e., 0 to 0.05%).

3.2 Add 50 µL of assay reaction mixture (from step 2) to each well of the ethanol standard, blank control, and test samples (see step 2, table 3) so that the total ethanol assay volume is 100 µL/well

Note: For a 384-well plate, add 25 µL sample, 25 µL of assay reaction mixture *per well.*

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

3.4 Monitor the fluorescence increase with 530-570 nm (optimal at 540) excitation and 590-600 nm emission using a fluorescence plate reader.
Note: The contents of the plate can also be transferred to a white clear bottom plate and read by absorbance microplate reader at the wavelength of 576±5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

4. Run 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.

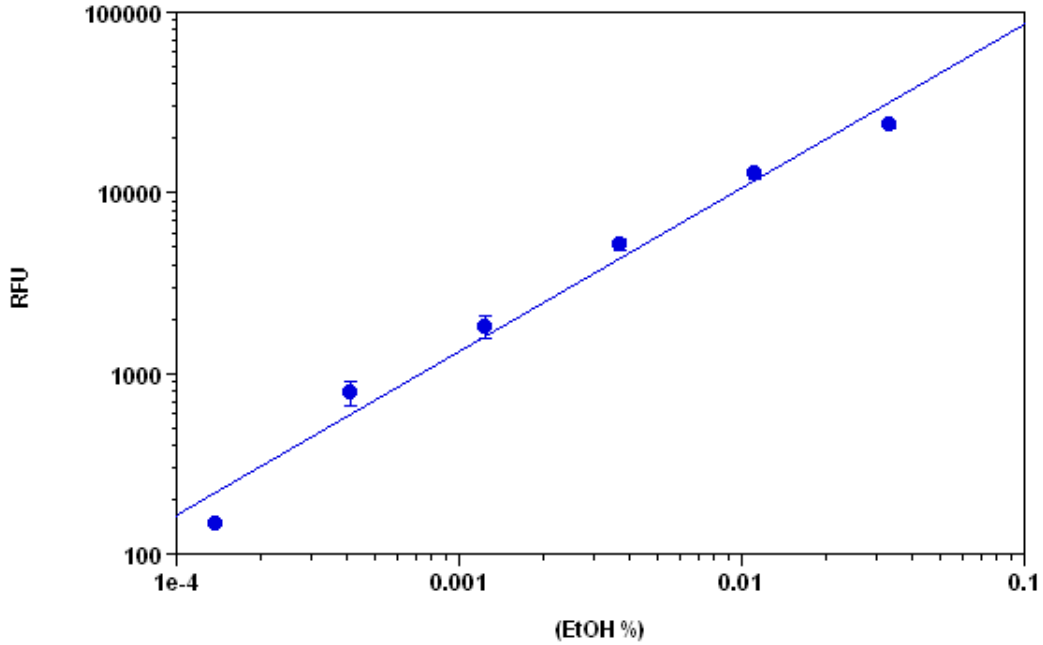

Figure 1. Ethanol dose response on 96-well black plate using a Gemini (molecular devices) measured with Amplite™ Ethanol Quantitation Kit. As low as 0.0003% of Ethanol can be detected with 15 minutes incubation time (n=3).

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.