Product Information

Ethanol Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2125-100 100 assays	Cap Color	Part Number
Ethanol Assay Buffer	25 ml	WM	K2125-C-1
Ethanol Probe (in DMSO,anhydrous)	200 µl	Red	K2125-C-2
Ethanol Enzyme Mix	1 vial	Green	K2125-C-3
Ethanol Standard (MW:46.07, 17.15N)	0.5 ml	Yellow	K2125-C-4

II. Introduction:

Ethanol (C_2H_5OH) is produced by the fermentation of sugars by yeasts and is present in alcoholic beverages. Low doses of alcohol is benefit for circulation while high doses of alcohol consumption may lead to various diseases. Quantitative determination of alcohol is of important significance in drug discovery, basic research, clinical studies and fermentation industry processes.

The Ethanol Colorimetric/Fluorometric Assay Kit provides a sensitive, fast and convenient way for detection of ethanol concentration in various biological samples such as plasma, serum, other body fluids, growth media, foods and beverages based on colorimetric and fluorometric method. Alcohol oxidase oxidizes ethanol to produce H_2O_2 which reacts with Ethanol Probe to yield fluorescence (Ex/Em = 535/587 nm) and color (λmax = 570 nm). The kit can detect 0.1 - 10 ppm alcohol (~ 10 - 800 nM).

III. Reagent Preparation:

Ethanol Probe: Ready to use as supplied. Warm to room temperature prior to use. Store at -20°C, avoid contamination with water, protect from light. Use within two months.

Ethanol Enzyme Mix: Add 220 µl Ethanol Assay Buffer to the Ethanol Enzyme Mix and mix well. Store at 4°C. Use within two months.

IV. Ethanol Assay Protocol:

Note:

Extreme care should be taken to ensure that no alcohol vapors are in the laboratory air where this assay is to be performed Alcohol vapors in the air will be rapidly absorbed by kit components resulting very high background making the kit unusable. Laboratory where equipment and solvents are standing or where alcohol is used to wipe down. Laboratory benches or equipment are inappropriate locations to perform this assay.

1. Standard Curve Preparations: For the colorimetric assay, add 50 µl of pure ethanol standard to 808.7 µl Ethanol Assay Buffer, mix well. Then take 10 µl of the dilution into 990 µl assay buffer to generate 10 nmol/µl of ethanol standard. Take 100 µl of the dilution into 900 µl assay buffer to generate 1mM (1nmol/ µl). Add 0, 2, 4, 6, 8, 10 µl to a series of wells in a 96 well plate and adjust the volume of each to 50 µl with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well ethanol Standard.

Note:

The fluorometric assay is 10-fold more sensitive than the colorimetric assay. For fluorometric measurement, dilute the ethanol standard to 0.1mM by adding 100 µl of the ethanol standard to 900 µl of Assay Buffer, mix well. Then add 0, 2, 4, 6, 8, 10 µl to a series of wells and adjust volume of each to 50 µl with Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well ethanol Standard.

2. Sample Preparation: Samples can be diluted directly in Assay Buffer and tested. Biological samples such as serum (containing ~ 0.01 - 0.16% w/v) should be diluted 1:10 - 1:100 and volumes in the range of 10 - 30 µl used. For beverages which contain 100X more alcohol, correspondingly greater

dilutions should be used. We suggest making several dilutions of your sample so that the sample reading is within the standard curve range. Adjust the final volume to 50 µl using Assay Buffer.

3. Reaction Mix Preparation: Mix enough reagent for the number of assays performed: For each well, prepare a total 50 µl Reaction Mix containing:

Ethanol Assay Buffer	46 µl
Ethanol Probe	2 µl
Ethanol Enzyme Mix	2 µl

Note: For fluorometric assay, use 0.2 µl Ethanol Probe per well to reduce background.

4. Add 50 µl of the Reaction Mix to all wells.

5. Incubate for 60 minutes at room temperature or 30 minutes at 37°C protected from light.

6. Measure O.D. 570 nm for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a micro-plate reader.

7. Correct background by subtracting the background value derived from the 0 ethanol control from all samples (The background reading can be significant and must be subtracted from sample readings). Calculate ethanol concentrations of the test samples from the standard curve, multiplied by the dilution factor.

C = Sa/Sv nmol/µl or mM

Where: Sa is sample amount from the Standard Curve (nmol).

Sv is sample volume added into the sample well (µl).

Ethanol molecular weight: 46.07 g/mol.

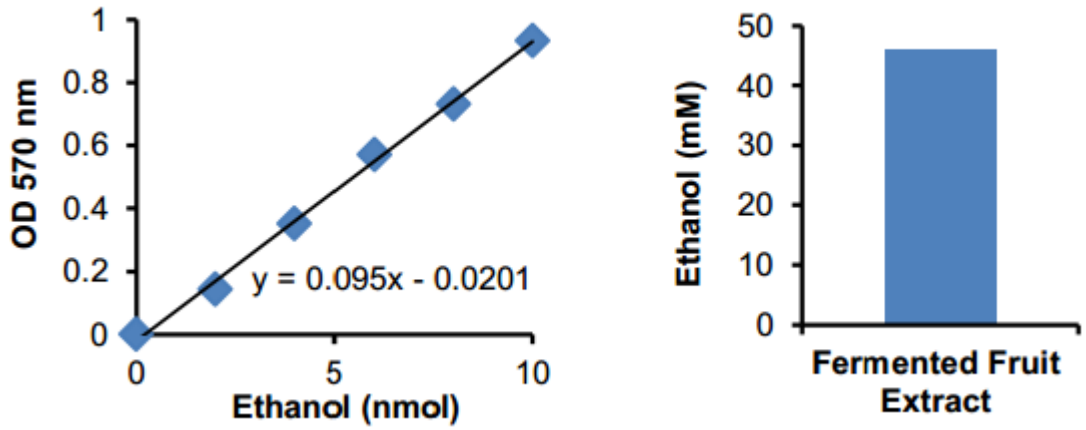

Figure: a) Ethanol Standard Curve. b) Measurement of Ethanol in fermented fruit extract (Cherry extract, 5 µl, 50X diluted). Fruit extract was treated with Carrez Clarification Reagent Kit for protein precipitation, spin filtered and diluted for the assay. Assay was performed following the kit protocol.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates

Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d atasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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