Ethanol Colorimetric/Fluorometric Assay Kit

(Catalog #K620-100; 100 assays; Store at -20°C)

I. Introduction:

Alcohol (ethanol C_2H_5OH) is among the most widely consumed drinks. Low doses of alcohol may help circulation while heavy alcohol consumption may lead to various forms of disease. Quantitative determination of alcohol finds applications in basic research, drug discovery, clinical studies and fermentation industry processes. BioVision's Ethanol Assay Kit provides a simple, rapid, and sensitive method for accurate quantification of ethanol concentration in a variety of biological samples such as serum, plasma, other body fluids, foods, beverages and growth media. Alcohol oxidase oxidizes ethanol to generate H_2O_2 which reacts with our probe to generate color (λ_{max}= 570 nm) and fluorescence (Ex/Em = 535/587 nm).. The kit detects 0.1-10 ppm alcohol.

II. Kit Contents

Components	Volume	Cap color	Part Number
Ethanol Assay Buffer	25 ml	WM	K620-100-1
Ethanol Probe (in DMSO,anhydrous)	200 µl	Red	K620-100-2A
Ethanol Enzyme Mix	1 vial	Green	K620-100-4
Ethanol Standard (MW:46.07, 17.15N)	0.5 ml	Yellow	K620-100-5

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 (ETHANOL, METHANOL, AND PROPANOL) 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 IN VERY HIGH BACKGROUND MAKING THE KIT UNUSABLE. LABORATORIES WHERE HPLC 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 50µl of pure ethanol standard to 808.7µl 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 50µl 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 10-30µl 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:

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

   ***Note:** For fluorometric assay, use 0.2 µl Ethanol Probe per well to reduce background.
4. Add 50µl 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

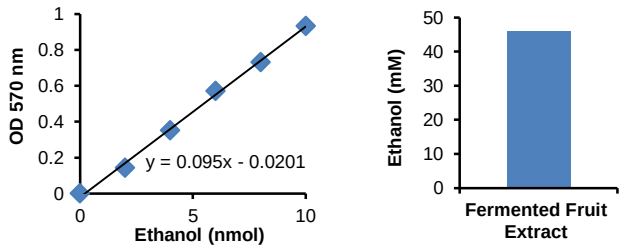

Figure: a) Ethanol Standard Curve. b) Measurement of Ethanol in fermented fruit extract (Cherry extract, 5 ul, 50X diluted). Fruit extract was treated with Carrez Clarification Reagent Kit (K809) for protein precipitation, spin filtered and diluted for the assay. Assay was performed following the kit protocol.

RELATED PRODUCTS:

Glucose Assay Kit	Lactate Assay Kit
Uric Acid Assay Kit	L-Amino Acid Quantification Kit
NAD/NADH Quantification Kit	ADP/ATP Quantification Kit
Cholesterol Assay Kit	ATP Cell viability Assay Kit
Quick Cell Proliferation Assay Kit	Apoptosis Assay Kits & Reagents

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		