Ethanol

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1236

(Version 1.5B)

Detection and Quantification ofEthanolContentin Urine, Serum, Plasma, Saliva and Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Alcoholic drinks are among the daily consumed beverages. Studies have shown heavy alcohol consumption may lead to various forms of liver diseases and to increased mortality rates. Quantitative determination of alcohol (ethanol, C2H5OH) has applications in basic research, drug discovery, clinic studies and in the alcoholic industry.

Ethanol Colorimetric Microplate Assay Kit is based on alcohol dehydrogenase catalyzed oxidation of ethanol, in which the formed NADH is coupled to the formazan chromogen. The intensity of the product color, measured at 450 nm, is proportionate to the ethanol concentration in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Reaction Buffer	10 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Coenzyme	Powder x 1	-20 °C, keep in dark
Dye Reagent A	Powder x 1	4 °C, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
Standard (200μg/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be200/100/50/25/12.5/6.25/3.125μg/L.

Enzyme: Briefly centrifuge prior to opening. Add 1 mlReaction Buffer to dissolve before use.Store at 4 °C for 2-3 days or -20 °C for1 monthafter reconstitution.

Coenzyme: Briefly centrifuge prior to opening. Add 1 mlReaction Buffer to dissolve before use.Keep in dark and store at 4°C for 1 week or -20 °C for 2 months.

Dye Reagent A: Add 9 ml distilled water to dissolve before use, mix.Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For liquid samples

Detect directly, or dilute with distilled water.

VI. ASSAY PROCEDURE

Warmall reagentsto room temperature before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	60 µl	60 µl	60 µl
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled Water	--	--	20 µl
Enzyme	10µl	10µl	10µl
Coenzyme	10µl	10µl	10µl
Mix, cover the plate adhesive strip, keep at room temperature for 5 minutes.			
Dye Reagent A	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl
Mix, keep at room temperature for 5 minutes, record absorbance measured at 450nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-Intercept}{Slope}\times n(\mu g/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu g/L)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

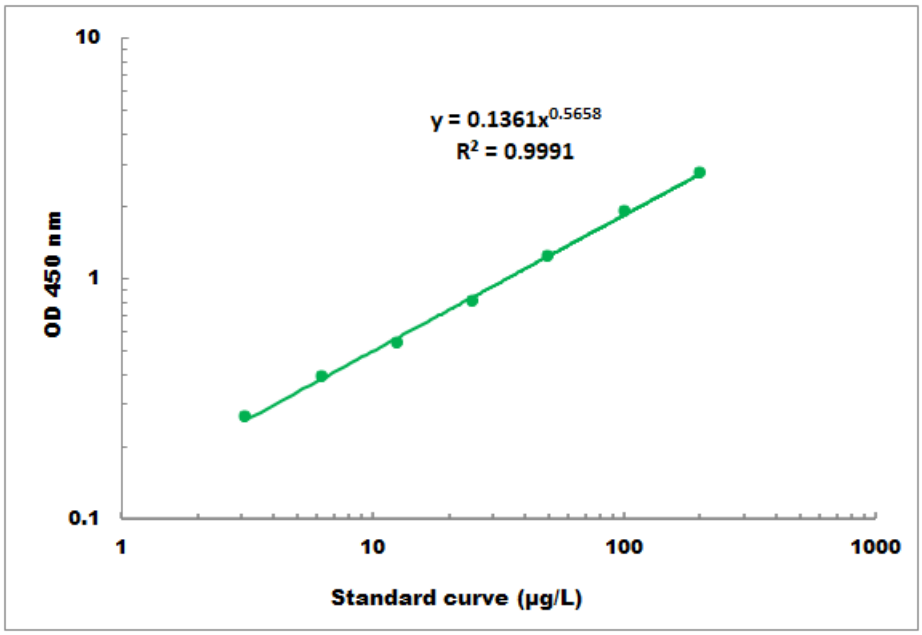
$C_{Standard}$: the standard concentration, μg/L

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 2µg/L - 200µg/L