Ethanol Microplate Assay Kit

User Manual

Catalog # CAK1236

(Version 1.4B)

Detection and Quantification of Ethanol Content in Urine, Serum, Plasma, Saliva and Other biological fluids Samples.

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 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-Well Microplate	1 plate	
Reaction Buffer	10 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Coenzyme	Powder x 1	-20 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard (200 μg/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Reaction Buffer to dissolve before use.

Coenzyme: add 1 ml Reaction Buffer to dissolve before use.

Dye Reagent A: add 9 ml distilled water to dissolve before use, mix, store at 4°C.

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. SAMPLE PREPARATION

1. For liquid samples

Detect directly, or dilute with Distilled water.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	60 µl	60 µl	60 µl
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled Water	--	--	20 µl
Enzyme	10 µl	10 µl	10 µl
Coenzyme	10 µl	10 µl	10 µl
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:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

$$\text{Ethanol } (\mu g/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

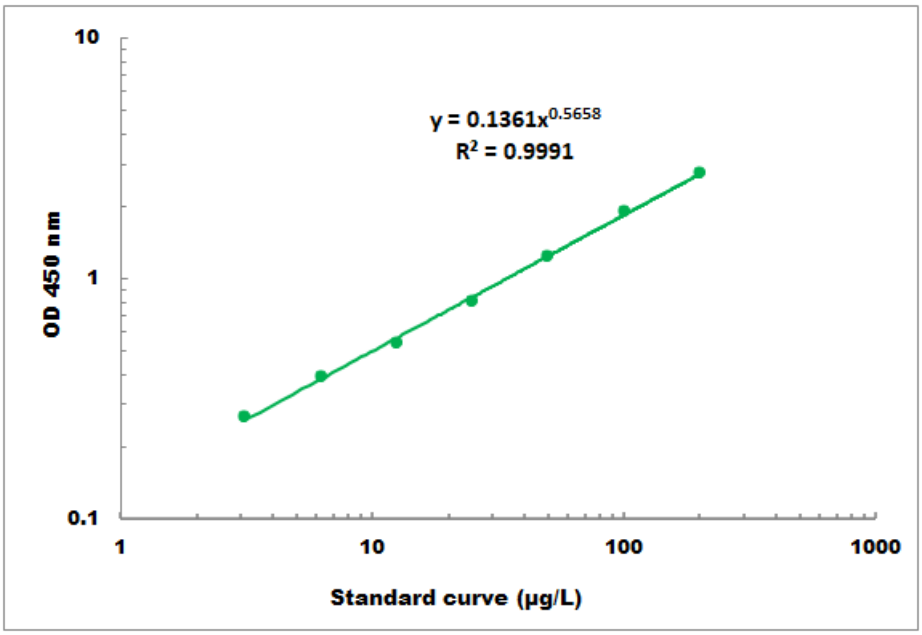
$C_{Standard}$: the standard concentration, 200 μg/L = 0.2 μg/ml;

$V_{Standard}$: the volume of standard, 20 μl = 0.02 ml;

V_{Sample}: the volume of sample, 20 μl = 0.02 ml.

VII. TYPICAL DATA

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



Detection Range: 2 μg/L - 200 μg/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES