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BIOSCIENCES

Ethanol Microplate Assay Kit

User Manual

Catalog # FTA0213

(Version 1.2A)

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 (MTT) chromogen. The intensity of the product color, measured at 570 nm, is proportionate to the ethanol concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	5 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Coenzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 1	4 °C
Standard (10 mg/L)	1 ml x 1	4 °C
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Note:

Enzyme: add 1 ml Distilled water to dissolve before use.

Coenzyme: add 1 ml Distilled water to dissolve before use.

Dye Reagent: add 10 ml Distilled water to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 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
Sample	30 µl	--	--
Standard	--	30 µl	--
Distilled water	--	--	30 µl
Reaction Buffer	50 µl	50 µl	50 µl
Enzyme	10 µl	10 µl	10 µl
Coenzyme	10 µl	10 µl	10 µl
Mix, keep at room temperature for 5 minutes.			
Dye Reagent	100 µl	100 µl	100 µl
Mix, keep at room temperature for 5 minutes, record absorbance measured at 570nm.			

Note:

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

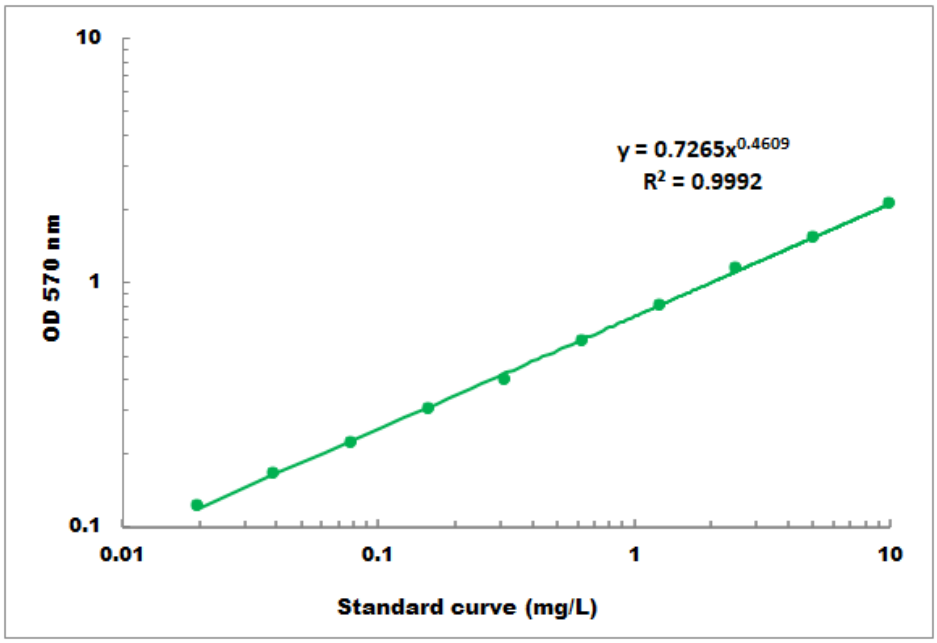
2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VI. CALCULATION

Subtract blank OD from the standard OD values and plot the OD against standard concentrations. Use the standard curve to determine the sample concentration.

VII. TYPICAL DATA

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



Detection Range: 0.01 mg/L - 10 mg/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