

Ethanol Fluorometric Assay Kit

(Catalog #TBS2095; 100 assays; store at -20°C)

DESCRIPTION

Quantitative determination of alcohol or ethanol finds many applications in clinical studies and research, and winery. Studies have shown heavy alcohol consumption may lead to various forms of liver diseases and to increased mortality rates. Tribo™ Ethanol Colorimetric Assay Kit provides a rapid, simple, reproducible, and sensitive tool for assay ethanol in plasma, serum, urine, and other bio-samples. The ethanol assay uses the alcohol oxidase-peroxide reaction for the determination of ethanol concentrations. The fluorescence of the reaction product at Ex/Em=530/590nm is directly proportional to the ethanol concentration in the sample.

APPLICATIONS

Direct Assays: ethanol in serum, plasma, urine, and other bio-samples.

KEY FEATURES

Sensitive and accurate. Use 10 μ L samples. Detection ranges 0.0002-0.01 vol % Ethanol in 96-well plate assay.

Simple and high-throughput. Simple procedure; takes less than 30 minutes. Kit is designed to be a robust method.

KIT CONTENTS

Assay Buffer 15 mL	Ethanol Standard 1 mL
Probe 60 μ L	Enzyme 110 μ L

STORAGE AND HANDLING

Store kit at -20°C. Shelf life of six months. Protect from light. Allow Reagent to warm to room temperature before use. Briefly centrifuge vials prior to opening.

ASSAY PROTOCOL

Ensure the Reagent is at room temperature before use. Keep samples and enzyme on ice during the assay. It is recommended that all standards and samples be duplicated in the assay.

1. Sample Preparations:

Serum, Plasma, other body fluid, or cell culture supernatant can be measured directly by a series of dilutions of the sample (1/2; 1/4; 1/8). Solid samples, such as tissues, can be first homogenized and extracted with PBS with a tissues/PBS ratio of 1:8 (1 hr at 4°C) followed by centrifugation at 10,000g. The Clear supernatants then can be measured as described for liquid samples. Add 10 μ L test samples directly into 96-well clear plate.

2. Standard Curve Preparations:

Mix 10 μ L 0.1% vol Ethanol standard with 90 μ L assay buffer to make 0.01% vol Ethanol standard, then 2 fold series dilute 40 μ L of 0.01 vol % Ethanol Standard with assay buffer to 0.05, 0.025, 0.0125, 0.006, 0.003, 0.0015 & 0 μ M. Transfer 10 μ L series diluted std into a 96-well plate.

3. Working solution: Prepare enough working solution by mix 90 μ L Assay Buffer with of 1 μ L Enzyme, 0.5 μ L probe for each reaction. Transfer 90 μ L working solution to each well containing the Standard and test samples. Tap plate lightly to mix. Incubate at room temperature for 20 minutes, protect from light.

4. Read plate at Ex530/Em590nm in a fluorescence plate reader.

5. Calculation:

a. Average the RFU values of replicate wells of each Ethanol standard and test samples. Subtract the average RFU value of the blank (0 μ M standard) from the averaged RFU values from all standards and samples.

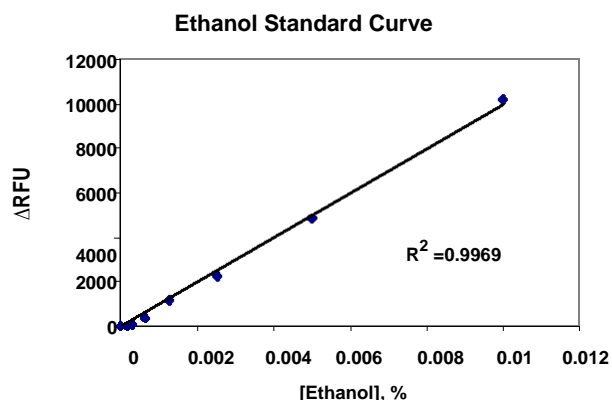
b. Make a standard curve by plotting Δ RFU values from each Ethanol standards as a function of Ethanol concentration. Calculate the concentration of Ethanol in samples using the equation obtained from the linear regression of the standard curve.

$$\text{Ethanol} = (\text{RFU}_{\text{sample}} - \text{RFU}_{\text{blank}}) / \text{Slope} (\%)$$

Where: RFU_{sample} and RFU_{blank} are fluorescence values of the sample and buffer.

If unknown sample results over standard curve range, dilute sample in assay buffer. Repeat the assay; multiply the results by the dilution factor *n*.

Note: 0.01 vol % ethanol equals 1.7 mM or 7.85 mg/dL.



Standard Curve in 96-well plate.

RELATED PRODUCTS:

ATP Colorimetric/Fluorometric Assay Kit (#TBS2010)
 ADP Colorimetric/Fluorometric Assay Kit (#TBS2020)
 ADP/ATP Ratio Assay Kit (#TBS2015)
 Glucose Colorimetric Assay Kit (#TBS2080)
 Glucose Fluorometric Assay Kit (#TBS2085)
 Ethanol Colorimetric Assay Kit (#TBS2090)