Tribioscience

Ethanol Assay (Colorimetric/ Fluorometric)

One-step Sensitive Quantitation for Ethanol or Alcohol in Serum, Plasma, Urine and Other bio-samples

Catalog Number	Kit Size
TBS2090-100	100 assays
TBS2090-200	200 assays

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 uses a single wording reagent to combine the alcohol oxidase reaction and color reaction into one step. The change in color intensity of the reaction product at 570 nm or fluorescence intensity at λex/em = 530/585 nm is directly proportional to ethanol concentration in the sample. The fluorometric assay is more sensitive than the colorimetric assay.

APPLICATIONS

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

KEY FEATURES

Flexible: Suitable for colorimetric and fluorometric methods.

Accurate: Use 50 µL samples. Detection ranges 0.2-20 mM in 96-well plate for colorimetric assay and 0.02-10 mM for fluorometric assay.

Simple and high-throughput: One-step procedure: just load-incubate-Read. The kit can be used for a robust method.

Time saving: Less than 30 minutes

KIT CONTENTS

Component	TBS2090-100	TBS2090-200
Assay Buffer	10mL	20 mL
Red Probe	0.25mL	0.5 mL
Ethanol Standard (17mM)	0.5 mL	1 mL
Enzymes	0.25 mL	0.5 mL

STORAGE AND HANDLING

Store kit at -20°C. Shelf life of 1year. Protect from light. Shipment with blue ice.

FLOROMETRIC PROTOCOL

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

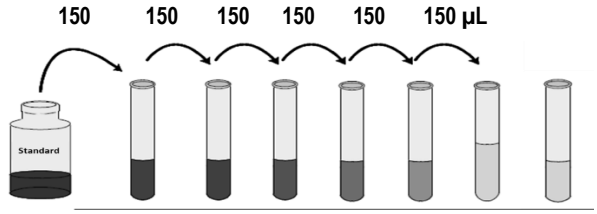
Sample Preparations:

Serum, Plasma, other body fluid, or cell culture supernatant can be measured directly by a series of dilutions of the sample (1/2, ¼, or 1/8). Solid samples, such as tissues, can be first homogenized and extracted with PBS (tissues/Ethanol ratio of 1:8) for 1 hr at 4°C, followed by centrifugation at 10,000g for 10min. The clear supernatants then can be measured as described for liquid samples. Add 50µL test samples directly into 96-well clear plate.

Standard Curve Preparations:

1. Label 1.5mL tube from Std2 to 8. As below the diagram. The provided Standard is directly used as Std1 (17mM).
2. Add 150uL of 1x Assay Buffer to Std2 to 8.
3. Take 150µL Standard solution to Std2, then make 2x series dilution in Std3 through 7. Std8 is 1x Assay Buffer alone as a standard 0. The standard concentration range is 17, 8.5, 4.25, 2.125, 1.06, 0.5, 0.25 & 0 mM.

Fig.1: Diagram of Standard Preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)		150	150	150	150	150	150	150
Addition		Std1	Std2	Std3	Std4	Std5	Std6	
Addition (µL)		150	150	150	150	150	150	
Final Conc (mM)	17	8.5	4.25	2.125	1.06	0.5	0.25	0

Work Solution

Mix 48 µL Assay Buffer with of 1 µL Enzymes, and 1µL probe for 50uL of each well.

Assay Procedures

1. Add 50 µL of standard or sample to each well of a black microplate in duplicate manner *(Note: the black microplate is for fluorescence detection)*.
2. Add 50 µL work solution to each well containing the Standard and test samples. Tap plate lightly to mix.
3. Incubate at 37°C for 30 minutes, protect from light.
4. Measure the fluorescence using a microplate reader, equipped for excitation in the range of 530-560nm and emission detection at ~ 590nm. *(Note: Because the assay is continuous (not terminated), fluorescence or absorbance may be measured at multiple time points to follow the kinetics of the reactions).*

Ethanol Assay (Colorimetric/ Fluorometric)

COLORIMETRIC PROCEDURE

The colorimetric assay is similar to the fluorometric assay. But its sensitivity is much less than the fluorometric assay. The linear detection range is 0.2 to 20 mM of ethanol. Prepare the standards using fluorometric Procedure to obtain standards at 17, 8.5, 4.25, 2.125, 1.06, 0.5, 0.25 & 0 mM.

1. Transfer 50 µL standards, samples into separate wells of a 96-well plate.
2. Add 50 µL Working Reagent (see fluorometric Procedure), tap plate to mix. Incubate 30 min at 37°C.
3. Read OD value at 570 nm (550-585 nm).

Calculation

Subtract the blank value (0 µM Standard) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the ethanol concentration of the Sample using the equation obtained from the linear regression of the standard curve.

Ethanol = N x (R_{sample}- R_{blank})/Slope (mM)

Where: Rsample and Rblank are optical density or fluorescence intensity readings of the sample and blank, respectively. N is the sample dilution factor.

Note1: If unknown sample results is over standard curve range, dilute samples with assay buffer, and repeat the assay.
Note2: 0.1% (Volume) ethanol = 17mM or 78.5mg/dL.

TYPICAL DATA

The typical data is provided for only demonstration references shown in Fig.2 and Fig.3.

Fig2. Ethanol Colorimetric Assay

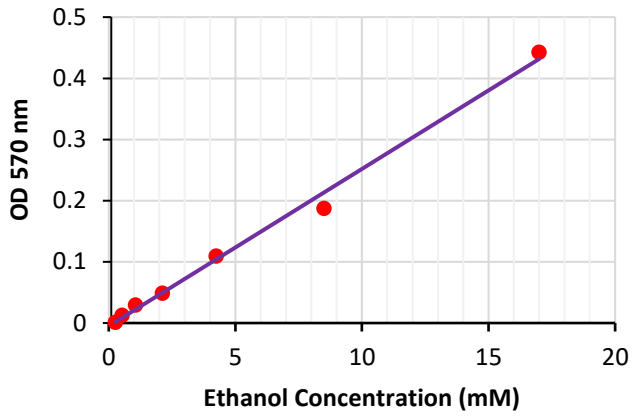
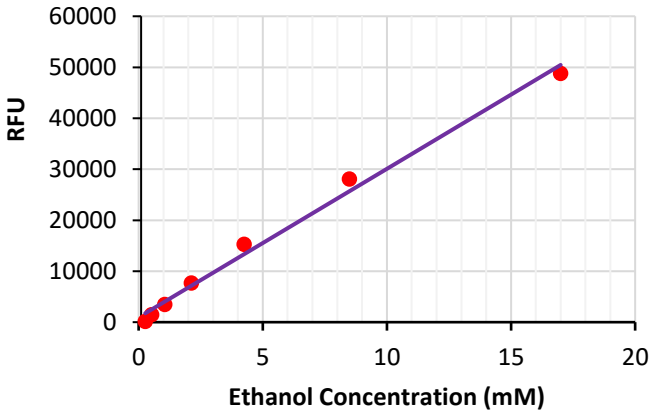


Fig3. Ethanol Fluoremetric Assay



RELATED PRODUCTS

Cell Viability Assay Kits (TBS2001)
ATP Colorimetric/Fluorometric Assay (TBS2010)
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
Glucose Oxidase Colorimetric/Fluorometric Assay (TBS2088)

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