

Ethanol Assay Kit (Colorimetric)

LS-K299-100 (100 Tests) • Store at -20°C



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.

Simple, direct and automation-ready procedures for measuring ethanol concentration are very desirable. LSBio's ethanol 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 565 nm, is proportionate to the ethanol concentration in the sample.

Key Features

- Sensitive and accurate. Detection limit 0.0008 vol % (140 µM or 8 ppm), linearity up to 0.1% ethanol in 96-well plate assay.
- Convenient. The procedure involves adding working reagent, incubating for 30 min and stopping reaction. No 37°C heater is needed.
- High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

Applications

- Direct Assays: ethanol in serum, plasma, urine and saliva samples.
- Pharmacology: effects of drugs on alcohol metabolism.

Components

Component	K299-100
	100 Tests
Assay Buffer	10 mL
NAD Solution	1 mL
Enzyme A	Lyophilized
Enzyme B	120 µL
MTT Solution	1.5 mL
Enzyme Buffer	150 µL
Standard (1% Ethanol)	1.5 mL
Stop Reagent	12 mL

Materials Not Supplied

Pipetting (multi-channel) devices. Clear flat-bottom 96-well plates (e.g. Corning Costar) and plate reader.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

Ethanol Assay Kit (Colorimetric)

LS-K299-100 (100 Tests) • Store at -20°C



Assay Procedure

Reagent Preparation:

Reconstitute Enzyme A by adding 120 µL Enzyme Buffer to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down. Store reconstituted Enzyme A at -20°C and use within 1 month.

Assay Procedure

1. Calibration Curve. Prepare 0.1% alcohol Premix by mixing 25 µL 1% Standard and 225 µL distilled water. Dilute standard as follows. Transfer 10 µL standards into wells of a clear flat-bottom 96-well plate.

No	Premix + H ₂ O	Vol (µL)	Ethanol (%)
1	100µL + 0µL	100	0.10
2	60µL + 40µL	100	0.06
3	30µL + 70µL	100	0.03
4	0µL + 100µL	100	0

Samples: add 10 µL sample per well in separate wells. IMPORTANT: saliva samples should be diluted 10-fold in PBS prior to assay.

2. Reaction. For each well of reaction, prepare Working Reagent by mixing 80 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B, 2.5 µL NAD and 14 µL MTT. Fresh reconstitution is recommended.

Add 90 µL Working Reagent per well quickly. Tap plate to mix briefly and thoroughly. Incubate 30 min at room temperature. Add 100 µL Stop Reagent per well. Tap plate to mix.

3. Read optical density at 565 nm (520-600nm).

Calculations

Subtract blank (water, #4) from OD values for the standard wells. Plot Standard Curve (ΔOD vs Standard ethanol concentrations) to determine the slope. Sample ethanol concentration is calculated,

$$[\text{Ethanol}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Slope}} \times n \quad (\%)$$

where OD_{SAMPLE} and OD_{BLANK} are the OD_{565nm} values of the sample and blank (water, #4). n is the dilution factor (n = 10 for saliva).

Note: if the sample ethanol concentrations are higher than 0.1%, dilute sample in distill water and repeat this assay. Multiply the results by the dilution factor.

Conversions: 1 vol % ethanol equals 170 mM or 785 mg/dL.

General Considerations

1. This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough. Use of multi-channel pipette is recommended.
2. The following substances interfere and should be avoided in sample preparation: ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%).

FOR RESEARCH USE ONLY! Not for use in humans.

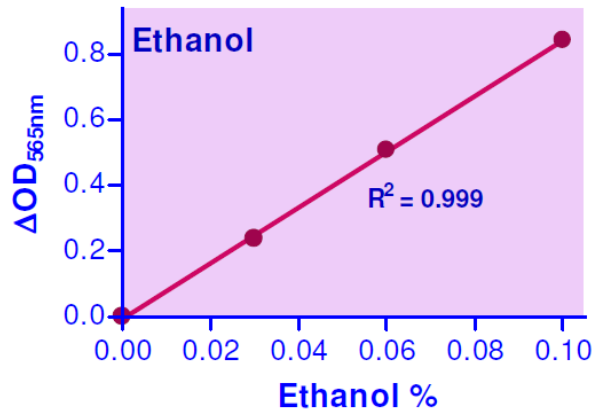
LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121

www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

Ethanol Assay Kit (Colorimetric)

LS-K299-100 (100 Tests) • Store at -20°C

Sample Data



Standard Curve in 96-well plate assay

Version: V.08.09.2018

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com