Datasheet for ABIN2345130

Alcohol Assay Kit (Fluorometric)

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Overview

Quantity:	100 tests
Application:	Biochemical Assay (BCA)

Product Details

Detection Method:	Fluorometric
Sensitivity:	15 µM
Characteristics:	Alcohol Assay Kit measures primary alcohols by an enzymatic, oxidation reaction, producing hydrogen peroxide which reacts with the kit's Fluorometric Probe (Ex. 530-560 nm/Em. 585-595 nm). The Alcohol Assay Kit is a simple, fluorometric assay that quantitatively measures the alcohol concentration (primary alcohols only) in various samples using a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, standards and unknown samples. The kit contains an ethanol standard and has a detection sensitivity limit of ~15 µM (0.00007 % w/v). Notes: 1) This kit can detect various primary alcohols and is not ethanol specific. Each alcohol will produce different sensitivity limits with different reaction rates (see Table 2). 2) This kit is not suitable for urine samples.
Components:	1. Ethanol Standard : One 500 µL vial of pure ethanol (MW 46.07, 17.15N) 2. 10X Assay Buffer : Three 1.5 mL vials 3. 100X Enzyme Mixture : One 100 µL vial 4. 200X Fluorometric Probe : One 55 µL amber vial 2
Material not included:	1. Standard 96-well fluorescence black microtiter plate 2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips 3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips 4. Multichannel micropipette reservoir 5. Fluorescence microplate reader capable of reading excitation in the 530-560 nm range and

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emission in the 585-595 nm range

Target Details

Background:	Alcohols can be found in various products including antiseptics, solvents, combustion fuels, and preservatives. However, the most commonly used alcohol (ethanol) has been consumed in beverages for thousands of years. Potential long-term effects of ethanol consumption include liver disease, cardiac conditions, pancreatitis, diabetes, and cancers.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Simple assays that quantitatively measure the alcohol concentration (primary alcohols only) in various samples using a 96-well microtiter plate format and is not ethanol specific • Each kit provides sufficient reagents to perform up to 100 assays, including blanks, standards and unknown samples
Reagent Preparation:	• 10X Assay Buffer should be thawed/maintained at 4 °C during assay preparation. Precipitation may be visible after thawing, mix well to dissolve the precipitate. The solution is stable for 1 week at 4 °C. For longer term storage, the solution should be aliquoted and frozen at -80 °C to avoid multiple freeze/thaws. • 1X Assay Buffer: Dilute the 10X Assay Buffer Concentrate with deionized water. Stir to homogeneity. . • Ethanol Standard and 100X Enzyme Mixture should be thawed/maintained at 4 °C during assay preparation. All are stable for 1 week at 4 °C. For longer term storage, each should be aliquoted and frozen at -80 °C to avoid multiple freeze/thaws. • 200X Fluorometric Probe should be thawed/maintained at room temperature during assay preparation. Any unused material should be aliquoted and frozen at -80 °C to avoid multiple freeze/thaws.
Sample Preparation:	• Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4 °C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80 °C for storage. Plasma must be diluted before assaying (1:20 to 1:100 in 1X Assay Buffer). • Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant 4 without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80 °C for storage. Serum must be diluted before assaying (1:20 to 1:100 in 1X Assay Buffer). • Saliva: Samples should be tested immediately or frozen at -80 °C for storage. Saliva must be

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	diluted before assaying (1:20 to 1:100 in 1X Assay Buffer). • Urine: This kit is not recommended for urine samples.
Assay Procedure:	Each ethanol standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed. 1. Add 10 µL of the diluted ethanol standards or samples to the 96-well fluorescence microtiter plate. 2. Maintain all components/mixtures at 4 °C. According to Table 3 (below), prepare the desired volume of Reaction Mixture (based on the # of tests) in the following sequence: a. In a clean tube, add the appropriate volume of deionized water. b. To the water add the corresponding volume of 10X Assay Buffer. Mix well. c. Next, add the corresponding volume of 100X Enzyme Mixture. d. Finally, add the corresponding volume of 200X Fluorometric Probe. Mix well and immediately use. Note: Reaction Mixture will appear slightly pink in color. This is normal background and should be subtracted from all absorbance values. Deionized 10X Assay 100X Enzyme 200X Total Volume # of Tests in Water (mL) Buffer (mL) Mixture (µL) Fluorometric of Reaction 96-well Plate Probe (µL) Mixture (mL) (90 µL/test) 8.850 1 100 50 10 100 4.425 0.5 50 25 5 50 2.213 0.25 25 12.5 2.5 25 Table 3. Preparation of Reaction Mixture 4. Transfer 90 µL of the above Reaction Mixture to each well (already containing 10 µL of ethanol standard or sample). 5. Cover the plate wells to protect the reaction from light. 6. Incubate at 37 °C for 30 minutes. 7. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-560 nm range and for emission in the 585-595 nm range. 8. Calculate the concentration of ethanol within samples by comparing the sample fluorescence to the standard curve. Negative controls (without ethanol) should be subtracted. 5
Restrictions:	For Research Use only

Handling

Storage:	-80 °C
Storage Comment:	Store the entire kit at -80°C. Avoid multiple freeze/thaws by aliquoting. The Fluorometric Probe is light sensitive and should be maintained in amber tubes.