Product Manual

Alcohol Assay Kit (Colorimetric)

Catalog Number

STA-620	**100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

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.

Cell Biolabs' Alcohol Assay Kit measures primary alcohols by an enzymatic, oxidation reaction, producing hydrogen peroxide which reacts with the kit's Colorimetric Probe (absorbance maxima of 570 nm).

The Alcohol Assay Kit is a simple, colorimetric 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 ~30 µM (0.0001 % w/v).

Notes:

1) *This kit can detect various primary alcohols and is* <u>*not*</u> *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.*

Related Products

1. STA-375: Uric Acid/Uricase Assay Kit
2. STA-378: Creatinine Assay Kit
3. STA-390: Total Cholesterol Assay Kit
4. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
5. STA-398: Free Glycerol Assay Kit (Colorimetric)

Kit Components

1. <u>Ethanol Standard</u> (Part No. 262001): One 500 µL vial of pure ethanol (MW 46.07, 17.15N)
2. <u>10X Assay Buffer</u> (Part No. 262002): Three 1.5 mL vials
3. <u>100X Enzyme Mixture</u> (Part No. 262003): One 100 µL vial
4. <u>50X Colorimetric Probe</u> (Part No. 238401): One 200 µL amber vial

Materials Not Supplied

1. 96-well microtiter plate
2. Microplate reader capable of reading at 570 nm

Storage

Store the Colorimetric Probe at -20ºC and all other kit components at -80ºC. For convenience the Colorimetric Probe may be frozen at -80ºC to keep it with the other kit components. Avoid multiple freeze/thaws by aliquoting. The Colorimetric Probe is light sensitive and should be maintained in amber tubes.

Preparation of Reagents

- 10X Assay Buffer should be thawed/maintained at 4ºC during assay preparation. Precipitation may be visible after thawing; mix well to dissolve the precipitate. The solution is stable for 1 week at 4ºC. For longer term storage, the solution should be aliquoted and frozen at -80ºC 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 4ºC during assay preparation. All are stable for 1 week at 4ºC. For longer term storage, each should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- 50X Colorimetric Probe should be thawed/maintained at room temperature during assay preparation. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.

Preparation of Ethanol Standard

- To prepare the ethanol standards, first perform a dilution of the stock Ethanol Standard in deionized water. Prepare only enough for immediate use (e.g., Add 11.7 µL of Ethanol Standard to 988.3 µL deionized water). This solution has a concentration of 200 mM. Use this 200 mM ethanol solution to prepare standards in the concentration range of 0 µM – 2000 µM by further diluting in 1X Assay Buffer (e.g., Add 5 µL of 200 mM ethanol solution to 495 µL 1X Assay Buffer - see Table 1 below). Ethanol diluted solutions and standards should be prepared fresh.

Standard Tubes	200 mM Ethanol Standard (µL)	1X Assay Buffer (µL)	Final Ethanol Standard (µM)	Final Ethanol Standard (% w/v)
1	5	495	2000	0.0117
2	100 of Tube #1	100	1000	0.0059
3	100 of Tube #2	100	500	0.0029
4	100 of Tube #3	100	250	0.0015
5	100 of Tube #4	100	125	0.0007
6	100 of Tube #5	100	62.5	0.00036
7	100 of Tube #6	100	31.25	0.00018
8	0	100	0	0

Table 1. Preparation of Ethanol Standards

Preparation of Other Alcohol Standards

- This kit will detect various alcohols and is not ethanol specific. However, in the assay, relative reaction rates differ between alcohol substrates. If your sample(s) primarily contain a particular alcohol, the appropriate alcohol standard curve should be created and used during sample determination. See Table 2 for relative reaction rates.
  Note: Assay optimization may be required.

Alcohol Substrate	Relative Reaction Rate
Methanol	1.0
Propargyl alcohol	0.9
Ethanol	0.82
Propanol-2-ene	0.81
n-Butanol	0.67
2-Chloroethanol	0.66
n-Propanol	0.43
2-Methoxyethanol	0.40
2-Cyanoethanol	0.30
Isobutanol	0.21

Table 2. Relative Reaction Rates of Alcohol Substrates

Preparation of Samples

- 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 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 diluted before assaying (1:20 to 1:100 in 1X Assay Buffer).
- Urine: This kit is not recommended for urine samples.

Assay Protocol

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 microtiter plate.
2. **Maintain all components/mixtures at 4ºC.** 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 50X Colorimetric 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 Water (mL)	10X Assay Buffer (mL)	100X Enzyme Mixture (µL)	50X Colorimetric Probe (µL)	Total Volume of Reaction Mixture (mL)	**# of Tests in 96-well Plate (90 µL/test)**
8.7	1	100	200	10	**100**
4.35	0.5	50	100	5	**50**
2.175	0.25	25	50	2.5	**25**

Table 3. Preparation of Reaction Mixture

3. Transfer 90 µL of the above Reaction Mixture to each well (already containing 10 µL of ethanol standard or sample).
4. Cover the plate wells to protect the reaction from light.
5. Incubate at 37ºC for 30 minutes.
6. Read absorbance in the 540-570 nm range on a microplate reader.
7. Calculate the concentration of ethanol within samples by comparing the sample absorbance to the standard curve. Negative controls (without ethanol) should be subtracted.

Example of Results

The following figures demonstrate typical Alcohol Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

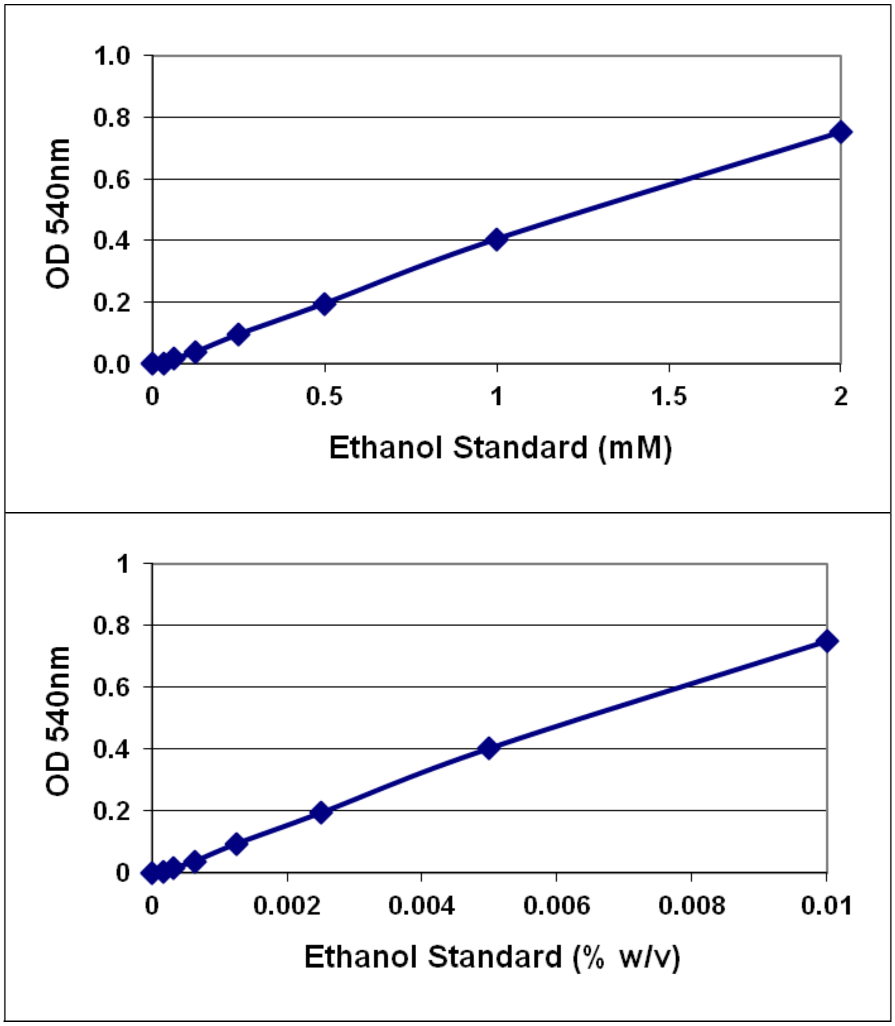

Figure 1: Alcohol Assay Standard Curve. Ethanol standard curve was performed according to the Assay Protocol, data plotted in mM (left) and % w/v (right).

References

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Recent Product Citations

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