



Ethanol test -alcohol industry.

For use on manual or automated spectrometers.

This enzymatic ethanol test is designed to accurately measure ethanol (ethyl alcohol) in commercial beverages to an accuracy of +/- 0.1%

ETHANOL Catalog Number C701-0B, consisting of :-

Ethanol Reagent R1: C701-03 (2 x 26 mL)

Ethanol Reagent R2: C701-04 (2 x 5.2 mL)

Ethanol Standard 2.4% v/v: C701-22 (2 x 1.5 mL)

StatFax® Analyzer: StatFax 4500

Intended Use

For the determination of the level of Ethanol (i.e. the alcohol percentage) in a sample of beer.

Precautions

Avoid contact of reagent with skin and eyes. Should skin contact occur, wash affected area with plenty of cold water. Should eye contact occur, rinse with a saline solution. **Never** pipette reagent by mouth.

Method Principle

The Catachem Ethanol procedure is based on the affinity of an enzyme Alcohol Dehydrogenase from bacteria to catalyze the conversion of Ethanol to Acetaldehyde with the concomitant reduction of NAD to NADH. The increase in absorbance at 340 nm is directly related to the concentration of Ethanol in the sample.

Alcohol dehydrogenase

Ethanol + NAD \longrightarrow Acetaldehyde + NADH

Ethanol R1 (Sample diluent)

Each liter contains:

Buffer, Surfactants, Stabilizer and nonreactive ingredients

Ethanol R2 (Activator)

Buffer

Alcohol dehydrogenase 400,000 U/L

NAD 9 mM

Surfactants, Stabilizers and nonreactive ingredients



Precautions

Handle this reagent using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH.** Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage And Stability

Store the Ethanol reagents at 2-8°C. When stored as directed, the reagents are stable until expiration date stated on the label.

Both reagent components are in a stabilized liquid format and are used as is. After uncapping the bottle each reagent is stable for at least 45 days if re-capped and stored in the refrigerator when not in use.

Sample Collection and Preparation

Using the graduated cylinder, fill to the 50mL mark with deionized or distilled water (the bottom of meniscus should be at the 50 mL mark). To this, add 1mL (1000 µL) of beer fresh from the bulk and mix thoroughly. This is the diluted beer sample that will be used in the assay.

Required Materials

In addition to StatFax analyzer:

- Glass Test Tubes
- 50mL graduated cylinder
- Three pipettes: a 10-100 µL that will be adjusted to 10 µL, a 200 µL pipette and one 1000 µL pipette. Please reference the insert included on how to properly use the pipettes when running this assay. (For simplicity and accuracy, we use the 10µL pipette to thoroughly wash the sample into the test reagent. This is not normal laboratory practice when using pipettes hence the need for this note)
- Disposable plastic pipette tips
- Catachem Ethanol Reagents

Expected Values

The range of expected values determined for this method is 3-9% alcohol.

Linearity

Under the noted test conditions and 1/51 sample dilution the assay is linear to 14 % v/v Ethanol.

Limit of Quantification (LOQ)

The assay is accurate to a level of 0.1% Ethanol v/v %

Procedure using StatFax 4500 (Awareness Technology) manual spectrometer.

With this assay you will use three pipettes: a 10-100 μL adjusted to a volume of 10 μL , a 200 μL pipette and one 1000 μL pipette. The 10-100 μL pipette will be pre-adjusted to read 10 μL , this should always be checked before performing the test. Catachem's Ethanol Standard is 2.4% ethanol by a volume/volume(v/v) ratio. This acts as the calibrator in this test.

Please reference the insert included on how to properly use a pipette.

1. Plug in the StatFax®.
2. Turn on the StatFax® using the switch located on the back-right hand side of the instrument.
3. Fill the graduated cylinder to the 50mL mark with deionized or distilled water (the bottom of meniscus should be at the 50 mL mark). To this, add 1mL (1000 μL) of fresh beer and mix thoroughly. This will be called the Diluted Beer Sample and will be used in the assay.
4. Obtain at least four test tubes; one for each Test Sample, one for the Standard and one for the Blank.
5. Place the four empty test tubes into the slots in the incubation block of the StatFax®.
6. Using the 1mL (1000 μL) pipette, add 1mL of Ethanol Reagent R1 to each of the test tubes. Label one as "Blank", one as Standard and the remaining tubes as "Test Sample 1", "Test Sample 2" and so on.
NOTE: Running tests in duplicate or triplicate and averaging the results will provide a more accurate answer.
7. Agitate or invert the bottle of Ethanol Standard 2.4% v/v to ensure it is mixed thoroughly.
8. Using the 10 μL pipette, add 10 μL of the Ethanol Standard 2.4% v/v into the test tube labeled "Standard".
NOTE: One test tube will contain no sample as it is used as the "Blank".
9. Using the 10 μL pipette, add 10 μL of the Diluted Beer Sample into each test tube labeled "Test Sample" which already contains the R1 Reagent.
10. Using the 200 μL pipette add 200 μL of Ethanol Reagent R2 to all of the test tubes.
11. Mix the test tubes by manual agitation or by covering and inverting.
12. Let the test tubes sit for 5 minutes in the slots in the incubation block on the analyzer.
13. On the StatFax® using the designated stylus:
 1. Select "Run Test".
 2. Select "Beer".
 3. Select "Yes".
 4. It will ask if you want to print the full header, click "YES".
 5. It will ask you if you want to use the stored blank, click "NO".
 6. Place the test tube labeled Blank in the slot to be read.
 7. Wait for the screen to display, "Remove Blank".
 8. A graph will appear on the screen, click "Discard".
 9. Place the test tube labeled Standard (containing R1, R2 and the 10 μL of the standard) in the slot to be read.
 10. When the screen displays "Remove the Standard" do so and a new graph will appear on the screen. Accept this graph. (This will act as the new calibration).
 11. "Insert Sample". Place your test tube containing the Test Sample in the slot. The value obtained will be the amount of Ethanol (i.e. percent alcohol by volume) in the sample.
 12. Remove the tube and insert any remaining Test Samples until all of the samples have been read.
 13. When all your samples have been read, click "Quit" at the bottom of the screen.
14. Turn off StatFax® with the switch on the back-right hand side of the instrument and unplug.
15. Remove all test tubes from the StatFax®. Rinse these test tubes with water followed by a rinse with deionized or distilled water. Keep test tubes upside down to drain dry for reuse.