

**Ethanol Determination in Beer****I. Introduction**

Ethanol concentrations in complex matrices such as beer can be measured directly and quickly using the YSI 2700 SELECT Biochemistry Analyzer. YSI's unique enzyme technology provides for rapid ethanol measurement. Measurements are virtually unaffected by color, turbidity, density, or pH.

When a sample is injected into the sample chamber, the ethanol diffuses into the membrane containing alcohol oxidase. The ethanol is immediately oxidized to hydrogen peroxide and acetaldehyde. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The current flow at the electrode is directly proportional to the hydrogen peroxide concentration, and hence to ethanol concentration.

Other alcohols can interfere with ethanol measurement by also oxidizing and producing a signal. Fortunately, most alcohols have much lower responses to alcohol oxidase than ethanol. One notable exception is methanol, which is over three times as sensitive to the enzyme as ethanol. Therefore, samples must be methanol-free.

**II. Materials and Setup**

- A. YSI 2700 SELECT Biochemistry Analyzer - equipped with a 2786 Ethanol Membrane and 2787 Ethanol Buffer.
- B. Ethanol standards (2.00 g/L, 3.20 g/L). Place the 2.00 g/L solution in Cal Station #2.
- C. Connect the 2700 SELECT to a suitable power source.
- D. Perform the instrument and membrane check described in the User's Manual (Section 3).
- E. Volumetric glassware (Class A recommended).
- F. The following instrument setup is recommended:

Sample size:	10 µL
Sample Station #	3
CalMethod	One Station

**Black Probe Parameters**

Chemistry	Ethanol
Unit	g/L
Calibrator	2.00 g/L
End Point	45 Sec
CalStation#	2

**White Probe Parameters**

Single Channel 2700	N/A
Dual Channel 2700	None

**Autocal Parameters**

Sample Error	ON
Temperature	1°C
Time	15 Min
Sample	5 Sam
Cal Shift	2%

**III. Method**

- A. Dilute sample to bring ethanol concentration into the linear range of the instrument, which is 0 to 3.20 g/L. Samples with 1.5 to 2.5 g/L ethanol will give the best results.
- B. Calibrate the 2700 SELECT with a 2.00 g/L ethanol standard solution.
- C. Check the linearity of the membrane at least once a day by injection of an ethanol linearity check solution (3.20 g/L). Refer to the User's Manual (Section 3) for specifications.
- D. Assay the sample prepared in A by aspiration into the 2700 SELECT. The linear range of the system is 0 to 3.20 g/L ethanol. If the value reported exceeds this, further dilution is required.
- E. Calibrate frequently as described in the User's Manual (Section 6).

**IV. Calculations**

To calculate % ethanol, multiply the reported value by the appropriate dilution factor.

Example: 5.00 mL of beer was diluted to 100 mL in a Class A volumetric flask. When assayed, the value reported was 1.56 g/L ethanol.

% Ethanol:

$$1.56 \text{ g/L} \times 0.100\text{L}/5\text{mL} = 0.03120\text{g ethanol/mL beer}$$

$$= 3.12\% \text{ (w/v)}$$

For a v/v result, divide the mass by the density of ethanol (0.789 g/mL at 20°C).

$$0.03120 \text{ g/mL} / 0.789 \text{ g/mL} = 0.0395\text{mL ethanol/mL beer}$$

$$= 3.95\% \text{ (v/v)}$$

**V. Samples Tested**

Ethanol concentrations (w/v) in several beers and wines were determined using YSI technology and Sigma Test Kit 332-BT.

	YSI	Sigma
Beer A	4.40%	4.39%
Beer B	3.75	3.76
Beer C	3.89	3.87
Beer D	3.74	3.79

**VI. Ordering Information****YSI No.**

2700	Biochemistry Analyzer
2786	Ethanol Membrane Kit
2390	Ethanol Standards Kit
2787	Ethanol Buffer Kit
2363	Potassium Ferrocyanide Test Solution
2392	NaCl Solution (for membrane installation)

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