

EN

ETHANOL

REF 984300

①	3 x 16 ml Reagent 1
②	3 x 4.5 ml Reagent 2

INTENDED USE

Reagent for photometric determination of low ethanol concentrations in homogenous liquid samples using automated Thermo Scientific Arena (20, 20XT and 30) or Gallery analyzer.

METHOD

Enzymatic test with Alcohol-Dehydrogenase (ADH). Method is performed at 37 °C, using 340 nm filter.

PRINCIPLE OF THE PROCEDURE

Ethanol + NAD⁺ <--ADH--> Acetaldehyde + NADH + H⁺

REAGENT INFORMATION

Reagent 1 (R1) 3 x 16 ml

Reagent 2 (R2) 3 x 4.5 ml

Note: Labels of reagents vials have two barcodes.

For Arena analyzers, turn the short barcode on the left side to the barcode reader.

For Gallery analyzers, turn the long barcode on the right side to the reading position of the reagent rack.

Concentrations

R1	Buffer	pH 9.0
	Sodium chloride	150 mmol/l
R2	Buffer	pH 6.6
	NAD	≥ 10 mmol/l
	ADH	≥ 50 kU/l

Precautions

The reagents contain sodium azide (< 0.1 %) as preservative. Do not swallow. Avoid contact with skin and mucous membranes. Take the necessary precautions for the use of laboratory reagents.

Preparation

The reagents R1 and R2 are ready-to-use.

Note: Check that there are no bubbles on the surface of the reagent when you insert vials in the analyzer.

Storage and Stability

Reagents in unopened vials are stable at 2...8 °C until the expiry date printed on the label. Do not freeze the reagents.

Due to the sensitivity of the assay, it is recommended to remove the reagents from the analyzer every day after the analysis. Store the opened reagents caps closed at 2...8 °C.

SAMPLES**Sample Type**

Food and other sample material, e.g. alcohol free and low alcohol beverages.

Sample concentration and Arena/Gallery application

All method related details are in the separate application note.

Arena and Gallery applications have a primary dilution of 1+19, this means that every sample is automatically first diluted with 1+19.

Primary dilution and the Dilution limits Low and High can be changed according to the example table below if needed.

Dilution 1+	Dilution limit (g/l)	
	Low	High
2	0.04	0.75
9	0.13	2.50
29	0.38	7.50
119	1.50	10

Sample preparation

If the sample has substances interfering the measurement, please handle it according to the following suitable preparation procedure:

- Pipetting of the sample must be always under the surface of the dilution solution.
- When filtering sample solution, the filtrate has not to drop, but rinse down the wall of the container.
- Use clear, colorless and practically neutral liquid samples directly.
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- Crush or homogenize solid or semi-solid samples.
- Weigh sufficient quantity of sample in a volumetric flask (take care of the measuring range), extract with water and filtrate, centrifuge or use Carrez clarification if necessary.
- Weigh sufficient quantity of fat containing samples into a volumetric flask (take care of the measuring range), extract with hot water. Cool to allow the fat to separate, make up the mark, place the volumetric flask in an ice bath for 15 min. and filter. Alternatively use Carrez clarification after extraction.
- Adjust acid samples to pH 8 - 9 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min.
- Treat strongly colored samples with polyvinylpyrrolidone (PVPP e.g. 1 g/100 ml Sample), or (if necessary adjusted to pH 9) measure instead with reagent blank each sample with sample blank.
- If possible, fill the sample tubes only half-full, and analyze the samples immediately in a batch run. Minimize also the sample on-board time.
- Note:** Storage samples caps on to avoid ethanol evaporation.
- Carrez clarification:** Weigh sufficient quantity of the sample into a 100 ml volumetric flask which contains approx. 60 ml dist. water. Subsequently carefully add 5 ml Carrez-I-solution (potassium ferrocyanide, 85 mmol = 3.60 g K₄[Fe(CN)₆] × 3 H₂O/100 ml), 5 ml Carrez-II-solution (zinc sulphate, 250 mmol = 7.20 g ZnSO₄ × 7 H₂O/100 ml) and 10 ml 0.1 M NaOH. Mix after each addition. Fill the volumetric flask with water to the mark, mix and filter.

Note: The assay is very sensitive.

Ethanol from air e.g. through disinfection/cleaning agents or beverages with higher ethanol concentration may cause creep reactions and false high results. It is recommended to run the assay in ethanol free air and minimize sample storage in the segments. It is recommended to fill half the sample tubes.

TEST PROCEDURE

See a separate application for the Arena or Gallery analyzer.

Materials required but not provided

Distilled water (aseptic and free of heavy metals) and general laboratory equipment.

Alcohol standard 500 mg/l (0.5 g/l) Cat no. 984384 (one level, water based) is not included in the kit.

Calibration

Water based Alcohol standard can be used or other. Ordering code for Ethanol standard is 984384. The standard is ready-to-use.

Quality Control

Use quality control samples at least once a day and after each calibration and every time a new bottle of reagent is used. It is recommended to use two level of controls. The control intervals and limits must be adapted to the individual laboratory requirements. The results of the quality control sample(s) should fall within the limits pre-set by the laboratory.

Available controls:

Alcohol standard can be used. If Alcohol standard is used also for calibration, an additional internal control is recommended to be used.

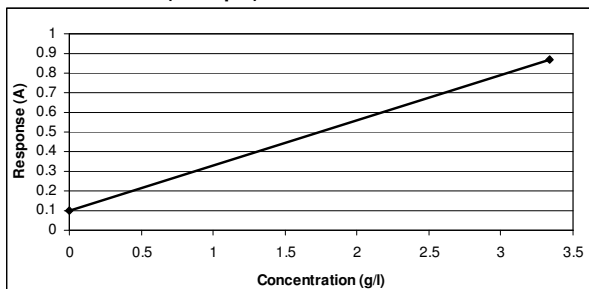
CALCULATION OF RESULTS

The results are calculated automatically by the analyzer using a calibration curve.

Conversion factors:

g/l x 21.6920 = mmol/l

mmol/l x 0.0461 = g/l

Calibration Curve (example)

Calibrator	Response (A)	Calc. conc. (g/l)
Water	0.098	0.000
Alcohol std	0.869	3.334

Calibration factor of this example is 0.216.

Note that the calibration curve is lot dependent.

LIMITATIONS OF THE PROCEDURE**Interference**

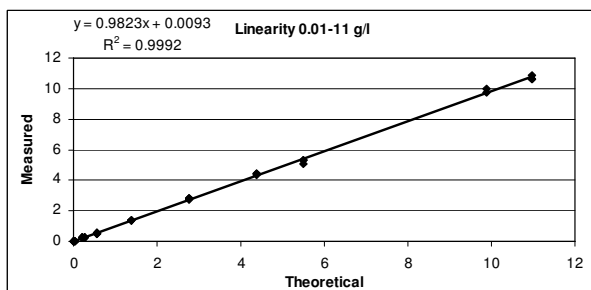
ADH oxidizes primary alcohols. Isopropanol and secondary butanol are slowly oxidized, while higher secondary and tertiary alcohols do not react.

MEASURING RANGE

The test has been developed to determine Ethanol concentrations within a measuring range from 0.01 to 10 g/l.

PERFORMANCE CHARACTERISTICS

The results obtained in individual laboratories may differ from the performance data given.

**Determination limit (=Test limit low)**

The determination limit is the lowest concentration that can be measured quantitatively. The determination limit (measured with water based standard solution) for this method is 0.01 g/l.

Precision**Arena analyzer**

	Mean 1.3 g/l		Mean 3.6 g/l	
	SD	CV %	SD	CV %
Within run	0.03	2.1	0.06	1.6

Gallery analyzer

	Mean 0.50 g/l		Mean 1.0 g/l		Mean 3.0 g/l	
	SD	CV %	SD	CV %	SD	CV %
Within run	0.007	1.5	0.010	1.0	0.030	1.0
Between run	0.009	1.9	0.012	1.2	0.042	1.4
Total	0.012	2.4	0.015	1.5	0.051	1.7

A precision study was performed using Gallery for 5 days, with the number of measurements being n = 50.

WASTE MANAGEMENT

Please refer to local legal requirements. Empty the cuvette waste bin daily immediately after the analysis.

OTHER REMARKS

All results must be verified by laboratory quality control samples.

Manufacturer does not warrant that the product is error-free or will accomplish any particular result. In no event shall the manufacturer be liable for special, incidental, indirect, punitive or consequential damages (including, but not limited to, loss of profits, loss of goodwill, loss of data or loss of use damages) arising out of the use or disposition of the products.

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Changes from previous version

Intended Use
Reagent Storage and Stability
Sample Type
Sample Preparation Note