

# Ethanol FS\*

Diagnostic reagent for quantitative in vitro determination of ethanol in serum and plasma on photometric systems

## Order information

Cat. No.	Kit size
1 0881 99 10 930	R1 4 x 20 mL + R2 2 x 10 mL
1 0881 99 90 314	R1 10 x 20 mL + R2 2 x 30 mL
1 0890 99 10 349	10 Ampoules with 1 mL Ethanol Standard 0.5 mg/mL
1 0910 99 10 349	10 Ampoules with 1 mL Ethanol Standard 1.0 mg/mL
1 0920 99 10 349	10 Ampoules with 1 mL Ethanol Standard 2.0 mg/mL
1 0930 99 10 349	10 Ampoules with 1 mL Ethanol Standard 3.0 mg/mL

## Summary

The determination of ethanol belongs to the most frequent analyses in the forensic and toxicological laboratory. It serves for the diagnosis of intoxications and poisonings particularly for emergency room patients.

## Method

Enzymatic UV test with alcohol dehydrogenase (ADH). The values of Ethanol Standard FS have been determined using a NIST SRM 2893 qualified Headspace gas chromatography/flame ionization detector (GC/FID).

## Principle



In the presence of NAD Ethanol is converted by alcohol dehydrogenase. The measured absorbance of the produced NADH is proportional to the ethanol concentration in the sample.

## Reagents

### Components and Concentrations

R1: Buffer	pH 9.0	300 mmol/L
R2: Buffer	pH 6.6	40 mmol/L
NAD		≥ 10 mmol/L
Alcohol dehydrogenase (ADH)		≥ 200 kU/L

### Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagents! The standards are stable up to the end of the indicated month of expiry, if stored at 15 – 25 °C. The content of the ampoules must be used immediately after opening since the indicated concentration changes due to evaporation. Ampoules have to be opened at the predetermined and marked breaking point. Opened ampoules of standards and controls can be used only once.

### Warnings and Precautions

- Reagent 1: Warning. H315 Causes skin irritation. H319 Causes serious eye irritation. P264 Wash hands and face thoroughly after handling. P280 Wear protective gloves/protective clothing/eye protection/face protection. P302+P352 If on skin: Wash with plenty of water/soap. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P332+P313 If skin irritation occurs: Get medical advice/attention. P337+P313 If eye irritation persists: Get medical advice/attention.
- The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
- Reagent 2 contains biological material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
- In very rare cases, samples of patients with gammopathy might give falsified results [5].

- Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.

### 6. For professional use only!

### Waste Management

Please refer to local legal requirements.

### Reagent Preparation

The reagents and the standards are ready to use.

### Materials required but not provided

NaCl solution 9 g/L  
General laboratory equipment

### Specimen

Serum and Plasma (heparin and EDTA) [3]

Due to alcohol evaporation, the sample container has to be filled as complete as possible, tightly closed, and should not stand open for longer than 5 min. In tightly closed sample tubes the stability in serum and plasma is 2 weeks at 20 – 25°C, 6 months at 4 – 8°C and 6 months at –20°C.

Do not use alcohol or volatile disinfectants during ethanol measurement.

Only freeze once! Discard contaminated specimens!

### Assay Procedure

*Application sheets for automated systems are available on request.*

Wavelength	376 nm (360 – 380 nm)
Optical path	1 cm
Temperature	37°C
Measurement	Against reagent blank

*The observance of exact measuring times and absolute equal treatment of all samples, standards and controls must be respected.*

	Reagent blank	Sample / Standard
Sample / Standard	-	10 µL
Dist. water	10 µL	-
Reagent 1	1000 µL	1000 µL
Mix and incubate 5 min. at 37°C. Read absorbance A1 then add:		
Reagent 2	250 µL	250 µL
Mix and incubate 5 min. at 37°C. Read absorbance A2 immediately.		

$$\Delta A = (A2 - A1) \text{ Sample / Standard}$$

### Calculation

One point or multi-point calibration

#### One-Point Calibration:

With standard 3.0 mg/mL (3.0 g/L);

Cat. No. 1 0930 99 10 349

$$\text{Ethanol [mg/mL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{Conc. Standard [mg/mL]}$$

#### Multi-Point Calibration

The ethanol concentration of unknown samples is derived from a calibration curve using a linear algorithm. The calibration curve is obtained with four standards at different levels (see order information) and NaCl solution (9 g/L) for determination of the zero value.

**Conversion factor**

Ethanol [g/L] x 21.7 = Ethanol [mmol/L]

Ethanol [g/L] (serum/plasma) x 0,8 = Ethanol‰ (whole blood)

**Controls**

For internal quality control DiaSys TruLab Ethanol should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruLab Ethanol	5 0900 99 10 349	10 x 1 mL

**Performance characteristics****Measuring Range**

The test has been developed to determine ethanol concentrations up to 3.5 g/L. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

**Specificity/Interferences**

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 60 mg/dL, lipemia up to 2000 mg/dL triglycerides, hemoglobin up to 1000 mg/dL, creatinine up to 250 mg/dL, glucose up to 2000 mg/dL, urea up to 2000 mg/dL and LDH up to 2000 U/L. For further information on interfering substances refer to Young DS [4].

**Sensitivity/Limit of Detection**

The lower limit of detection is 0.1 g/L.

**Precision (at 37°C)**

Intra assay n = 20	Mean [g/L]	SD [g/L]	CV [%]
Sample 1	0.51	0.01	1.67
Sample 2	0.98	0.02	1.95
Sample 3	1.99	0.01	0.66

Inter assay n = 20	Mean [g/L]	SD [g/L]	CV [%]
Sample 1	0.51	0.02	3.36
Sample 2	1.01	0.02	2.03
Sample 3	1.99	0.03	1.66

**Method comparison**

A comparison of DiaSys Ethanol FS (y) with a commercially available assay (x) using 30 samples gave following results:  
 $y = 1.0 x + 0.1$  g/L;  $r = 0.999$

**Reference Range [2]**

Ethanol is present in serum and blood only after ingestion.

0.3 – 1.2 g/L	6.5 – 26.0 mmol/L	Slowed reflexes, diminution of attention, judgment and control
1.2 – 2.5 g/L	26.0 – 54.3 mmol/L	Reduced visual acuity and increased reaction time
2.5 – 3.5 g/L	54.3 – 76.0 mmol/L	Muscular incoordination, decreased response to stimuli
> 3.5 g/L	> 76.0 mmol/L	Impairment of circulation and respiration, possible death

**Literature**

1. Thomas L. Clinical Laboratory Diagnostics. 1<sup>st</sup> ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 1168-1170.
2. William H., Porter Ph.D. Clinical Toxicology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3<sup>rd</sup> ed. Philadelphia: W.B Saunders Company; 1999. p. 922-923.
3. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1<sup>st</sup> ed. Darmstadt: GIT Verlag; 2001; p. 28-9.
4. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th. ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
5. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.

**Manufacturer**

DiaSys Diagnostic Systems GmbH  
 Alte Strasse 9 65558 Holzheim Germany