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Liquid Reagents – ready to use

ETHANOL

Enzymatic, UV
2 Reagents

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

REF	Kit Size	Content
D07810B	**1 x 1 L**	1 x 0,8 L R1 + 1 x 0,2 L R2
D07830	**5 x 50 mL**	4 x 50 mL R1 +1 x 50 mL R2
D07840	**5 x 25 mL**	4 x 25 mLR1 + 1 x 25 mL R2
D07850	**5 x 10 mL**	4 x 10 mL R1 + 1 x 10 mL R2
D67911	**5 x 50 mL**	4 x 50 mL R1 + 2 x 25 mL R2
D0447917	**5 x 50 mL**	4 x 50 mL R1 + 1 x 50 mL R2
DA0824	**5 x 20 mL**	4 x 20 mL R1 + 1 x 20 mL R2
DT1024	**5 x 20 mL**	4 x 20 mL R1 + 1 x 20 mL R2
DK0723	**5 x 50 mL**	4 x 50 mL R1 + 1 x 50 mL R2
DB0923	**2 x 100 mL**	2 x 80 mL R1 + 2 x 20 mL R2

Additionally offered:
Z05880 4 x 1 mL Ethanol Calibrator/Control Set

TEST PARAMETERS

Method:	Enzymatic, UV, Increasing Reaction
Wavelength:	376 nm (360 – 380 nm)
Temperature:	37°C
Sample:	Serum or plasma (heparin, EDTA)
Linearity:	up to 350 mg/dL (3.5 g/L)
Sensitivity:	The lower limit of detection is 10 mg/dL (0.1 g/L)

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.

TEST PRINCIPLE

$$\text{Ethanol} + NAD^+ \xleftrightarrow{ADH} \text{Acetaldehyde} + NADH + H^+$$

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

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1	
Buffer, pH 9.0	300 mmol/L
Stabilizers and preservatives	
Reagent 2	
Buffer, pH 6.6	40 mmol/L
NAD	≥ 10 mmol/L
Alcohol dehydrogenase (ADH)	≥ 200 kU/L

REAGENT PREPARATION

Reagents are ready to use.

REAGENT STABILITY AND STORAGE

Conditions:	Protect from light! Close immediately after use Do not freeze the reagents! Avoid contamination
Storage:	at 2 – 8°C
Stability:	up to the expiration date

SAMPLE STABILITY AND STORAGE

Serum and plasma (heparin and EDTA) [3]

Stability:	at 20 – 25 °C	2 weeks
(in tightly closed	at 4 - 8 °C	6 months
Sample tubes)	at -20 °C	6 months

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 minutes.
Don't use alcohol or volatile disinfectants during ethanol measurement!
Freeze only once! Discard contaminated specimens!

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

STANDARDS/CONTROLS

(not included in the kit – to be ordered separately)

Concentration:	0, 50, 100, 300 mg/dL
Storage:	2 – 8 °C
Stability:	up to the expiration date

Close immediately after use!

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Pipette into test tubes	Blank	Standard	Sample
Sample	-	-	10 µL
Standard	-	10 µL	-
Dist water	10 µL	-	-
Reagent 1	1000 µL	1000 µL	1000 µL
Mix and incubate 5 min at 37 °C. Read absorbance A1 against reagent blank, than add:			
Reagent 2	250 µL	250 µL	250 µL
Mix and incubate 5 min. at 37°C. Read absorbance A2 immediately. $\Delta A = (A2 - A1)$			

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

CALCULATION

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

UNIT CONVERSION

Ethanol [mg/dL] x 0.217 = Ethanol [mmol/L]
Ethanol [mg/dL] (plasma/serum) x 0.008 = Ethanol ‰
Ethanol [g/L] x 21.7 = Ethanol [mmol/L]
Ethanol in [g/L] (plasma/serum) x 0.8 = Ethanol ‰

REFERENCE RANGE [2]

Ethanol is present in serum and blood only after ingestion.

30 – 120 mg/dL (0.3 – 1.2 g/L)	Slowed reflexes, diminution of attention, judgment and control
120 – 250 mg/dL (1.2 – 2.5 g/L)	Reduced visual acuity and increased reaction time
250 – 350 mg/dL (2.5 – 3.5 g/L)	Muscular incoordination decreased response to stimuli
> 350 mg/dL (> 3.5 g/L)	Impairment of circulation and respiration, possible death

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

The test has been developed to determine ethanol concentrations up to 350 mg/dL (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.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 10 mg/dL (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

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	30 mg/dL
Bilirubin	60 mg/dL
Creatinine	250 mg/dL
Glucose	2000 mg/dL
Hemoglobin	1000 mg/dL
LDH	2000 U/L
Triglycerides	2000 mg/dL
Urea	2000 mg/dL

For further information on interfering substances refer to Young DS [4].

METHOD COMPARISON

A comparison between Dialab Ethanol (y) and a commercially available test (x) using 30 samples gave following results:
y = 1.00 x – 0.10 g/L; r= 0.999.

CALIBRATION

The assay requires the use of an ethanol standard.
We recommend the Dialab **Ethanol Calibrator/Control Set**..

QUALITY CONTROL

All controls with ethanol values determined by this method can be used.
We recommend the Dialab **Ethanol Calibrator/Control Set**.
Each laboratory should establish corrective action in case of deviations in control recovery.

AUTOMATION

Special adaptations for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

1. 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.
2. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. Reagent 2 contains biological material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
4. In very rare cases, samples of patients with gammopathy might give falsified results [5].
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
6. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
7. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

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3. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st 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.