ALCOHOL (ETHANOL)

Endpoint Enzymatic Determination of Alcohol in Serum, Plasma, whole Blood and Urine

5 x 10 ml **REF CP01-50**

INTENDED USE

Kit for quantitative determination of Alcohol (Ethanol) in serum, plasma and whole blood.

ASSAY PRINCIPLE

Ethanol in the presence of NAD + and ADH is converted into Acetaldehyde + NADH. The absorbance of NADH, measured spectrophotometrically at 340 nm, is proportional to the alcohol concentration of the sample.

REAGENTS

Kit composition:	**CP01-50**
REAGENT 1 (powder)	**5 vials**
Tris Buffer	
NAD^+, ADH	
Stabilizers and preservatives	
STANDARD (Std)	**1 x 2,5 ml**
Ethanol	

NOTE: standard concentration is printed on the vial label.

STABILITY: stored at 2-8°C, reagents are stable up to the expiration date on the label.

ADDITIONAL REAGENTS NOT INCLUDED IN THE KIT

Solution of trichloroacetic acid (TCA) 62.5 g/L.

NECESSARY ITEMS – NOT PROVIDED

Usual laboratory equipment: UV/VIS Spectrophotometer with temperature control; automatic micropipettes; Optical glass cuvettes or, alternatively, disposable ones in optical polystyrene; Saline solution.

PREPARATION OF WORKING REAGENT

Pipette exactly 10 ml of distilled water in a vial of Reagent 1.
Shake gently until complete dissolution.
Let the working reagent stabilize for 10 minutes.
STABILITY: at least 7 days at 2-8°C.

NOTE: discard any working reagent if turbid or if the absorbance at 340 nm is > 0.500.

SAMPLE

Serum, plasma, whole blood and urine (do not use alcohol swabs for blood collection).
Use heparin, potassium oxalate, EDTA, sodium citrate or sodium fluoride as anticoagulants.
STABILITY in whole blood:
2 days at 18-25°C, 2 weeks at 2-8°C and 4 weeks at -15°C.
Store samples tightly closed to avoid evaporation.

MANUAL ASSAY PROCEDURE

Wavelength:	340 nm
Optical path:	1 cm
Reading:	against blank reagent
Temperature:	37- 30°C - room temperature
Reaction time:	10 –15 –30 minutes
Linearity:	up to 300 mg/dl
Reagent/Sample:	300/1

Let reagents and samples reach room temperature before use.

PROCEDURE WITHOUT DEPROTEINIZATION

(sample: serum, plasma, urine)

Pipette in cuvette or test tubes labeled as it follows:
B/R: Blank Reagent, Std: Standard, S: Sample

	B/R	Std	S
Working reagent	3,0 ml	3,0 ml	3,0 ml
Demineralised water	10 µl		
Standard		10 µl	
Sample			10 µl

Mix accurately. Incubate for 10 minutes at 37°C, for 15 minutes at 30°C or for 30 minutes at room temperature. Read sample (As) and standard (Ast) absorbencies at 340 nm against blank reagent. The reaction is stable for 2 hours.

PROCEDURE WITH DEPROTEINIZATION

(sample: whole blood, icterical, hemolyzed or turbid serum or plasma)

Pipette in centrifuge test tubes:

	Std	S
TCA 62.5 g/L solution	1,8 ml	1,8 ml
Standard	200 µl	
Sample		200 µl

Close test tubes and shake vigorously. Wait for 5 minutes. Centrifuge for 5 minutes at 2000-3000 rpm (standard does not require centrifuge).
Pipette in cuvette or test tubes labeled as it follows:

	B/R	Std	S
Working reagent	3,0 ml	3,0 ml	3,0 ml
Demineralised water	100 µl		
Standard diluted with TCA		100 µl	
Supernatant			100 µl

Mix accurately. Incubate for 10 minutes at 37°C, for 15 minutes at 30°C or for 30 minutes at room temperature. Read sample (As) and standard (Ast) absorbencies at 340 nm against blank reagent. The reaction is stable for 2 hours.

CALCULATION

Calculate concentration in the sample using the following formulas:

Alcohol [g/L]= (As / Ast) x Std concentration [g/L]

Alcohol [mmol/L]= (As / Ast) x Std concentration [mmol/L]

REFERENCE VALUES

Ethanol concentration in:

WHOLE BLOOD:

mg/dl (mmol/L)	Symptoms
50-100 (10.9-21,7)	Flushing, slowing of reflexes, impaired visual activity
> 100 (>21,7)	Central nervous system depression (CNS)
> 400 (>86,8)	fatalities reported (i.e. respiratory failure)

SERUM and URINE: multiply by 1.2 - 1.3 the values shown for whole blood

NOTE: alcohol level can not be detectable in abstaining subjects.

PERFORMANCE CHARACTERISTICS

Sensitivity: the sensitivity of the method is about 10 mg/dl.
Sensitivity for 100 mg/dl of ethanol: about 0.430 ΔAbs.

Linearity: up to 300 mg/dl (65 mmol/L).
For higher values, dilute samples with saline solution and multiply the result by the dilution factor.

Within-run precision:

	Level 1	Level 2
Average (mg/dl)	41.2	108.3
DS	0.87	1.41
CV %	2.1	1.3

Between-run precision:

	Level 1	Level 2
Average (mg/dl)	41.6	109.5
DS	1.6	1.3
CV %	3.97	1.23

Correlation: correlation of FAR (Y) method with another kit available on the market (X) gave the following result:

Y = 1.0069 X – 0.21 r = 0.9987

Interferences:

- deprotein icterical, hemolyzed or turbid serum or plasma before the assay (see Procedure with deproteinization).
- urine: it is recommended to perform a blank sample (10 µl of sample + 3,0 ml of water) and deduct the blank sample absorbance from the sample absorbance.

Some alcohols interfere with the determination but react more slowly than ethanol. To avoid any interference, respect incubation time stated in the procedure.

NOTES

1. Statutory limits for alcohol concentration in blood to drive motor vehicles are different in function of the considered country.

WARNINGS AND PRECAUTIONS

REAGENT 1

WARNING
Reagent 1 is irritating for the eyes (H319). It can cause skin irritation (H315).
In case of contact with the eyes: rinse thoroughly for several minutes. If the irritation continues, see a doctor.
REAGENT 2 and STANDARD are not classified as dangerous. Avoid contact with the skin and ingestion.
Follow usual precautions when using chemical substances.

MANUFACTURER

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalogue number
	temperature limits
	use by
	caution
	consult accompanying documents

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