BIOLABO
www.biolabo.fr
MANUFACTURER:
BIOLABO SAS,
Les Hautes Rives
02160, Maizy, France

ALCOHOL ETHANOL

Reagent for quantitative determination of alcohol in human serum, plasma, whole blood or urines.

REF	99029	**R1**	10 x 10 mL	**R2** 1 x 5 mL
REF	99059	**R1**	2 x 100 mL	**R2** 1 x 10 mL

TECHNICAL SUPPORT AND ORDERS
Tel : (33) 03 23 25 15 50
Fax: (33) 03 23 256 256

CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1)

Most toxicologists consider ethanol to be the most often used and abused chemical substance. Consequently, the measurement of ethanol is one of the more frequently performed tests in toxicology laboratory. Although less frequently encountered, it is important to include methanol, isopropanol and acetone (a metabolite of isopropanol) in a test battery for alcohols for proper evaluation (gas chromatography analysis) of the acutely intoxicated patient.

PRINCIPLE (4)

Enzymatic method described by Gadsen R. H. and al. Reaction scheme is as follows:

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

The ratio of ADH and NAD^+/ Alcohol is maintained elevated so that equilibrium is reached relatively quickly. The conversion of ethanol to acetaldehyde proceeds rapidly. A “trapping agent” is used to drive the reaction to the right by complexion of acetaldehyde as it is formed.
The absorbance of NADH, proportional to alcohol concentration in the specimen, is measured by end-point reading at 340 nm.

REAGENTS COMPOSITION

Vial R1 **ENZYME COENZYME**

NAD^+ (Nicotinamine adenine dinucleotide phosphate)	≥ 2.4	mmol/L
ADH (Alcohol dehydrogenase)	≥ 25 000	IU/L
TRIS Buffer	pH 8.65 ± 0.1 at 25° C	
Stabiliser		
Preservatives		

Before reconstitution:
Xn, R22-32: Harmful if swallowed, Contact with acids liberates very toxic gas
S22-38: Do not breathe dust. In case of insufficient ventilation, wear suitable respiratory equipment

Once reconstituted: None

Vial R2 **STANDARD**

Ethanol: approximately 100 mg/dL (21.7 mmol/L)
The exact concentration is printed on the label of the vial R2.

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENT PREPARATION

REF 99029 (vial R1): Use a non-sharp instrument to remove aluminium cap.
Add promptly to the contents of the vial R1 the volume of demineralised water stated on the label.
Mix gently and wait for complete dissolution before using reagent (approximately 10 minutes).

STABILITY AND STORAGE

Store at 2-8°C well capped in the original vial and away from light.

- Standard (vial R2): transfer the requested quantity, well recap the vial and store at 2-8°C.
- When stored and used as described, unopened reagent (vial R1) and standard (vial R2) are stable upon expiry date stated on the label.
- Once reconstituted and free from contamination:
  Working reagent (vial R1) is stable for 7 days Discard any reagent (vial R1) if cloudy or if absorbance at 340 nm is > 0.500.

Don’t use working reagent (vial R1) after expiry date stated on the label of the kit.

SPECIMEN COLLECTION AND HANDLING (1) (2)

Urines. Serum, plasma, whole blood (alcohol swabs should not be used during blood specimen collection). Use heparin, Potassium oxalate, E.D.T.A., Sodium citrate or fluoride as anticoagulant.

- Stability in whole blood (without sodium fluoride as preservative): at 18-25° C up to 2 days, at 2-8° C up to 2 weeks, at –15° C up to 4 weeks.
- Stability in whole blood (with Sodium fluoride as preservative): at 18-25° C up to 2 weeks, at 2-8° C up to 3 months, at –15° C up to 6 months.

Specimens must be kept capped to avoid evaporative loss to the atmosphere.

INTERFERENCES (3)

Interferences studies performed on sera show no interference with Proceduren°1:

Interferent	Alcohol in (mg/dL)	Results
Ascorbic Acid	95	No interference up to 25 mg/dL
Total Bilirubin	96	No interference up to 418 µmol/L
Hemoglobin	90	No interference up to 189 µmol/L
Glucose	50.7	No interference up to 1000 mg/dL
Turbidity	90	No interference up to 0.308 abs (lactescence)

Higher icteric, hemolysed or cloudy plasmas or sera may be deproteinised before performing the assay (§ **MANUAL PROCEDURE**).

Several alcohols interfere with the determination but react more slowly than ethanol (respect incubation time stated in the procedure):

Substance	approximate % of reactivity
Ethanol	100
n-Butanol	28
Isopropanol	4
Methanol	0.3
Ethylene glycol	1.6
Acetone	0

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control.
3. Demineralised water for the reconstitution of the reagent.
4. TCA (Trichloro-acetic acid) 62.5 g/L.

CALIBRATION

- Standard provided in the kit (vial R2) measured in standardized conditions with enzymatic method and aqueous standard traceable to NERL Standard
- Or any calibrator traceable to a reference method or material.

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

To ensure good results, it is recommended to calibrate in the following cases:

1. When changing reagent batch.
2. After maintenance operations on the instrument.
3. When control values are out of range, even after using a new vial of fresh control.

QUALITY CONTROL

- Normal Control Ethanol/Ammonia/Bicarbonate REF 95013
- Pathological Control Ethanol/Ammonia/Bicarbonate REF 95023
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control and repeat the test.
3. If control is still out of range, use a new vial of calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

CLINICAL SIGNS (1) (2)

Ethanol concentration in WHOLE BLOOD:		States of alcoholic influence
mg/dL	**mmol/L**	
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)
URINES:	Values in post-absorbtive state are similar to those of serum	
SERUM:	Multiply by 1.2 to 1.3 the whole blood values.	

Alcohol level is virtually no detectable in abstaining subjects.
Not all individuals experience the same degree of CNS dysfunction at similar blood alcohol levels. The statutory limit of blood alcohol concentration for driving a motor vehicle is different in function of the considered country.

PERFORMANCES CHARACTERISTICS (4)

Within run N = 20	*Low level*	*High level*
Mean mg/dL	41.2	108.3
S.D. mg/dL	0.87	1.41
C.V. %	2.1	1.3

Between run N = 20	*Low level*	*High level*
Mean mg/dL	41.6	109.5
S.D. mg/dL	1.6	1.3
C.V. %	3.97	1.23

Detection limit: approximately 10 mg/dL.

Sensitivity for 100 mg/dL: approximately 0.430 Abs at 340 nm.

Comparison studies with a commercially available reagent (enzymatic method):

40 sera within 40 and 280 mg/dL have been evaluated with both reagents (linear regression):

y = 1.0069 x – 0.21 r = 0.9987

X (mg/dL)	Acceptable Inaccuracy (4)	Y calculated value	Observed Inaccuracy	Conclusion
100	+/-5	100	0	Passed
300	+/-9	302	2	Passed

LINEARITY

The assay is linear up to 300 mg/dL (65 mmol/L).

Above, dilute the specimen (1 + 4) with saline solution and re-assay taking into account dilution factor to calculate the result. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagent and specimens at room temperature.

Procedure n°1: Sera, plasmas, Urines (Without deproteinisation)

Pipette into 5 mL test tubes:	**Blank**	**Standard**	**Assay**
Working Reagent (vial R1)	3 mL	3 mL	3 mL
Demineralised water	10 µL		
Standard (vial R2)		10 µL	
Specimen			10 µL

Mix. Incubate for 10 minutes at 37° C or 15 minutes at 30° C or 30 minutes at room temperature.
Read absorbance at 340 nm (or Hg 334) against reagent blank.
The reaction is stable for 2 hours (see **§ INTERFERENCES**).

Note:

Urines: It is recommended to perform a specimen blank (10 µL specimen + 3 mL water, read against water). Then deduct the absorbance of specimen blank from the absorbance of assay (read against reagent blank).

Procedure n°2: whole blood, very icteric, hemolysed or cloudy sera or plasmas (With deproteinisation)

1-Supernatant preparation

Pipette in centrifuge tube:	**Standard**	**Specimen**
TCA Solution 62,5 g/L	1.8 mL	1.8 mL
Standard (vial R2)	200 µL	
Specimen		200 µL

Cap tubes. Mix vigorously. Let stand for 5 minutes.
Centrifuge for 5 minutes at 2000-3000 RPM. (Do not centrifuge Standard).

2- Assay

Then, pipette into 5 mL test tubes:	**Blank**	**Standard**	**Assay**
Working Reagent (vial R1)	3 mL	3 mL	3 mL
Demineralised water	100 µL		
TCA diluted Standard		100 µL	
Supernatant			100 µL

Mix. Incubate for 10 minutes at 37° C or 15 minutes at 30° C or 30 minutes at room temperature.
Read absorbance at 340 nm (or Hg 334) against reagent blank.
The reaction is stable for 2 hours (see **§ INTERFERENCES**).

Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 922-927.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 1344-1347.*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-251-to 3-253*
(4) *GADSDEN R.H., TAYLOR E.H., STEINDEL S.J. et al: Ethanol in Biological Fluids by Enzymic Analysis. In: Selected Methods of Emergency Toxicology. C.S. Frings, W.R. Faulkner, Eds. Vol 11. Selected Methods of Clinical Chemistry, Washington DC, AACC Press, 1986, p. 63-65*

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with