BIOLABO
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MANUFACTURER:
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ALT GPT (IFCC)

Reagent for quantitative determination of Alanine amino transferase activity (ALT) [EC 2.6.1.2] in human serum and plasma

REF LP80507	**R1** 4 x 30 mL	**R2** 1 x 30 mL
REF LP80607	**R1** 4 x 100 mL	**R2** 1 x 100 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (2)

ALT is present in very high amounts in liver and kidney, and in smaller amounts in skeletal muscle and heart. Although serum levels of both AST and ALT become elevated whenever diseases process affecting liver cells integrity, ALT is the more liver-specific enzyme.
A serum elevation of ALT activity is rarely observed in conditions other than parenchymal liver disease (cirrhosis, carcinoma, hepatitis, obstructive jaundice or liver stroke). Moreover, its elevation persists longer than do those of AST activity. Measurement of both AST and ALT has some value in distinguishing hepatitis from other parenchymal lesions.

PRINCIPLE (4) (5) (6)

Method developed by Wrobleski and La Due, optimised by Henry and Bergmeyer (following modified IFCC recommendations). Reaction scheme is as follows:

L- Alanine + 2-Oxoglutarate $\xrightarrow{\text{ALT}}$ Pyruvate + L-Glutamate

Pyruvate + NADH + H^+ $\xrightarrow{\text{LDH}}$ L-Lactate + NAD^+

The decrease in absorbance proportional to ALT activity in the specimen, is measured at 340 nm.

Absence of P_5P allows a better stability of working reagent.

REAGENTS COMPOSITION

R1	**BUFFER ENZYMES**	BUF ENZ **ALT**
L-Alanine	700	mmol/L
LDH	≥ 2500	UI/L
EDTA	6	mmol/L
Tris Buffer	135	mmol/L
pH à 30°C	7.50 ± 0.1	
Stabilizer		

R2	**COENZYME**	COENZ **ALT**
Tris Buffer	20	mmol/L
NADH	≤ 1.4	mmol/L
2-Oxoglutarate	80	mmol/L
Stabilizer		

According to 1272/2008 regulation (CLP), these reagents are not classified as dangerous

REAGENTS PREPARATION

Ready for use.

MATERIAL REQUIRED BUT NOT PROVIDED

1.Medical analysis laboratory equipment.
2.Spectrophotometer or Biochemistry Clinical Analyzer

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use (do not pipette with mouth).

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- 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.

STABILITY AND STORAGE

Stored away from light, well cap in the original vial at 2-8°C, reagent is stable when stored and used as described in the insert:

Unopened,

- Until the expiry date stated on the label of the Kit.

Once opened:

- Transfer requested quantity, well recap vials and store at 2-8°C,
- 2 separated reagents are stable at least 6 months without contamination
- Discard any cloudy reagent or if reagent blank is < 1.000 at 340nm.

SPECIMEN COLLECTION AND HANDLING (2) (7)

Unhemolysed serum. Do not use heparinised plasma.

ALT is stable in serum or plasma for:

- 24 hours at room temperature.
- 7 days at 2-8°C.

LIMITS (3) (6)

LDH contained in reagent allows, during pre-incubation step, the reduction of endogenous pyruvate which would positively interfere.
Elevated ALT level may involve NADH depletion during pre-incubation stage, which may lead to under-estimated results. In case of lipemic or icteric specimens, increased absorbance may mask this phenomenon. It's recommended to check these specimens diluted (1 + 4) in saline solution.

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

CALIBRATION

- REF 95015 BIOLABO Multicalibrator traceable *to ERM-AD454k*

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

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 | H2O Demineralized water | Biological hazard

Made in France
Latest revision: www.biolabo.fr
Revision: 08/02/2019

QUALITY CONTROL

- REF 95010 BIOLABO EXATROL-N Level I
- REF 95011 BIOLABO EXATROL-P Level II
- 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 serum and repeat the test
3. If control is still out of range, verify analysis parameters: Wavelength, temperature, specimen/reagent ratio, time counting, calibration factor
4. If control is still out of range, use a new vial of reagent and reassay
5. If control is still out of range, please contact BIOLABO technical support or your local Agent

EXPECTED VALUES (2)

UI/L	at 30°C	at 37°C
Newborns, Infants	9-32	13-45
Men	7-28	10-40
Women	5-25	7-35

Each laboratory should establish its own normal ranges for the population it serves.

PERFORMANCES at 37°C on KENZA 240TX

Linearity Range: between 10 and 390 IU/L

Detection limit: approx. 9 IU/L

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	19.9	55.6	185.7
S.D. IU/L	0.9	2.0	2.5
C.V. %	4.3	3.6	1.4

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	19.7	55.6	185.0
S.D. IU/L	1.0	2.5	5.0
C.V. %	4.9	4.6	2.7

Comparison studies with commercially available reagent:
Realised on human specimens (n=100) between 5 and 400 IU/L
y = 0.9900 x + 0.2592 r = 0.9985

Analytical sensitivity: approx. 0.0066 abs/min for 10 IU/L

Interferences:

Total bilirubin	Negative interference from 219 µmol/L
Direct bilirubin	No interference up to 420 µmol/L
Ascorbic acid	No interference up to 2500 mg/dL
Glucose	No interference up to 1060 mg/dL
Turbidity	Positive interference from 0.152 OD
Haemoglobin	Positive interference from 128 µmol/L

Other substances may interfere (see § Limits)

On the board stability: 2 separate reagents are stable 60 days.

Calibration Frequency: 30 days

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations.

PROCEDURE

Detailed Kenza 240TX procedure is available on request.

Wavelength: 340 nm
Temperature: 37°C
Let stand reagents and specimens at room temperature

	Automated analyzer	Manual procedure
Reagent 1	200 µL	800 µL
Reagent 2	50 µL	200 µL
Mix. Wait for 15 sec then add:		
Calibrator, Control or Specimen	25 µL	100 µL
Mix. After 60 sec, measure variation of absorbance per minute (ΔAbs/min) during 180 sec.		

Note:

1-Performances and stability data have been validated on KENZA 240TX and KENZA 450TX

2- With Manual Procedure on Spectrophotometer and on other biochemistry analyzers, performances and stability data should be validated by user

3- Applications proposal are available on request

CALCULATION

With Seric Muticalibrator:

$$\text{ALT Activity} = \frac{(\Delta\text{Abs/min) Specimen}}{(\Delta\text{Abs/min) Calibrator}} \times \text{Calibrator Activity}$$

With Theoretical Factor:

Activity (U/L) = ΔAbs/min x Factor

$$\text{Factor} = \frac{\text{VR} \times 1000}{6.3 \times \text{VE} \times \text{P}}$$

With:
VR = Total reactional volume (mL)
VE = Specimen volume (mL)
6.3 = Molar extinction coefficient for NADH at 340nm
P = Pathlength (cm).

Example, with Manual Procedure,
(Pathlength 1 cm, 37°C, 340 nm):

IU/L = (ΔAbs/min) x 1746

$$\mu\text{Kat/L} = \frac{\text{UI/L}}{60}$$

REFERENCES

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(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 64-67*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-6 to 3-16.*
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