BIOLABO
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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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Latest revision: www.biolabo.fr

CE

IVD

Made In France

I: corresponds to significant modifications

INTENDED USE

This reagent is designated for professional use in laboratory (manual or automated method).
I It allows the quantitative determination of alanine amino transferase (ALT) [EC 2.6.1.2] to screen its level in human serum and plasma.

GENERALITIES (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).

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 $\xrightleftharpoons{\text{ALT}}$ 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

R1 BUF ENZ **ALT**	Buffer Enzymes	
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 COENZ **ALT**	Coenzyme	
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

SAFETY CAUTIONS

- 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 or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENTS PREPARATION

Ready for use.

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.

MATERIAL REQUIRED BUT NOT PROVIDED

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

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with

QUALITY CONTROL

- REF 95010 EXATROL-N Level I
- REF 95011 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. Prepare a fresh control serum and repeat the test
2. If control is still out of range, use a new vial of fresh calibrator
3. If control is still out of range, use a new vial of reagent and reassay

If control is still out of range, please contact BIOLABO technical support or your local Agent.

REFERENCE INTERVALS (2)

	(IU/L) 37°C
New-borns, Infants	13-45
Men	10-40
Women	7-35

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

PERFORMANCES

On Kenza 240TX, 37°C, 340 nm

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

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)

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

On the board stability: 2 separate reagents are stable 30 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.

CALIBRATION

- REF 95015 Multicalibrator traceable *to ERM-AD454k*

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

I PROCEDURE

Manual method :

Let stand reagents and specimens at room temperature

Pipette in 1cm pathlength thermostated cuvette	
Reagent 1	800 µL
Reagent 2	200 µL
Bring at 37°C, then add:	
Calibrator, Control or Specimen	100 µL
Mix. Start a timer. Record initial absorbance after 60 sec at 340 nm. Record the absorbance again every minutes during 180 sec. Measure absorbance change per minute (∆Abs/min).	

1- Performances with manual procedure should be validated by user.
2- Kenza applications and other 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

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

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 = (∆Abs/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*
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