BIOLABO
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MANUFACTURER:
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ALT GPT (IFCC) Single vial

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

REF 80027	**R1** 20 X 10 mL	REF 80127	**R1** 8 x 30 mL	REF 80227	**R1** 10 x 125 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 $\xrightleftharpoons{ALT}$ Pyruvate + L-Glutamate

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ 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	**ALT (GPT) IFCC**	Reagent 1
2-Oxoglutarate	15	mmol/L
L-Alanine	500	mmol/L
LDH	≥ 1600	UI/L
NADH	≤ 0.18	mmol/L
Tris Buffer	100	mmol/L
pH at 30°C	7.50 ± 0.1	
Preservative		

Danger. Acute Tox. 2: H300 - Fatal if swallowed,
Aquatic Chronic 3: H412 - Harmful to aquatic life with long lasting effects

P264: Wash hands thoroughly after handling, P270: Do not eat, drink or smoke when using this product, P301+310: IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician, P330: Rinse mouth, P501: Dispose of contents/container in accordance with dangerous waste disposal regulations. Classification due to Sodium Azide < 1 %. For more details, refer to Safety Data Sheet (SDS)

Once reconstituted, working reagent is 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

- REF 80027 Use a non-sharp instrument to remove aluminium cap.
- Once opened, add promptly to the contents the amount of demineralised water indicated on the label.
- Mix gently until complete dissolution.

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 expiry date stated on the label.

Once reconstituted:

- Working reagent is stable for 60 days when free from contamination.
- Discard any reagent if cloudy or if absorbance at 340 nm is < 1.000.
- Don't use working reagent after expiry date.

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.

EXPECTED VALUES (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 17 and 350 IU/L

Detection limit: approx. 1.3 IU/L

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	36	47	202
S.D. IU/L	0.70	0.85	1.10
C.V. %	1.91	1.82	0.55

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	22.6	57.7	191.7
S.D. IU/L	0.61	1.03	5.22
C.V. %	2.7	1.8	2.7

Analytical Sensitivity: approx.. 0. 010 ΔAbs/min for 17 IU/L.

Comparison studies with commercially available reagent:

y = 0.9813 x – 0.6606 r = 0.9983

Interferences:

Turbidity	No interference up to 0.250 abs
Total bilirubin	Negative interference from 130 µmol/L
Ascorbic acid	No interference up to 2500 mg/dL
Glucose	No interference up to 1010 mg/dL
Haemoglobin	Positive interference from 434 µmol/L

Other substances may interfere (see § Limits)

On the board stability: 1 month

Calibration Stability: 1 month

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	1000 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

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 652-657*
(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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