ALT GPT – Instructions for use (IFU)

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R1: 4 x 50 mL - R2: 4 x 20 mL • REF A-R0200001001

R1: 3 x 18,5 mL – R2: 1 x 18,5 mL • REF R3330000008

IVD CE

INTENDED USE
Product for use in the quantitative determination in vitro of the GPT activity in human serum or plasma. The results of the test must always be interpreted in conjunction with the clinical context. For professional use only.

CLINICAL SIGNIFICANCE
Alanine aminotransferase (ALT/GPT) and aspartate aminotransferase (AST/GOT) are part of the group of aminotransferases or transaminases. These catalyze the reversible transformation of α-cheto-acids into aminoacids through the transfer of amino groups. AST and ALT are present in human plasma, bile, cerebrospinal fluid and saliva. In viral hepatitis and other forms of hepatic disease, the serum ALT level increases even before the appearance of clinical signs and pathological symptoms. The ALT activity can reach values 100-times higher than the upper reference range limit, although in most cases the increase corresponds to 20-25 times the normal values.

PRINCIPLE
In the presence of 2-oxoglutarate, alanine is transformed into pyruvate and glutamate by the GPT present in the sample. In the presence of NADH and Lactate-D-hydrogenase (LDH), Pyruvate is transformed into lactate and NAD. The consumption of NADH over a given period of time, determined at λ 340 nm, is proportional to the GPT concentration in the test sample.

Abbreviations:
ALT: Alanine aminotransferase
LDH: Lactate dihydrogenase
NADH: Reduced Nicotinamide-adenine dinucleotide
NAD+: Oxidated Nicotinamide-adenine dinucleotide

REAGENTES
A-R0200001001- R1: 4 x 50 – R2: 4 x 20 mL
Reagent 1: n° 4 vials x 47,0 mL ready for use
Reagent 2: n° 4 vials x 5,5 mL ready for use
R3330000008 - R1: 3 x 18,5 – R2: 1 x 18,5 mL
Reagent 1: n° 3 vials x 12,6 mL ready for use
Reagent 2: n° 1 vials x 4,0 mL ready for use

Concentrations

Reagent 1:		
	Conc.	**U.M.**
TRIS buffer pH 7.8 ± 0.2	110	mM
L-Alanine	550	mM
LDH	≥ 1320	U/L
2-Oxoglutarate	16.5	mM
Sodium azide	30.0	mM
Reagent 2:		
	Conc.	**U.M.**
TRIS buffer pH 10.2 ± 0.2	10.0	mM
NADH	2.60	mM
Sodium azide	30.0	mM

Precautions
Kit for professional laboratory use, used only by qualified and properly trained technical personnel, under the supervision of a doctor in charge of the laboratory. In addition to risk indications related to the active components, reagents may contain inactive components such as preservatives and detergents. The total concentration of these components is lower than the limits reported in the EC 1272/2008 Regulation and subsequent amendments and additions. However, it is recommended to handle reagents according to the standards of good laboratory practice

Reports of serious incidents
In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements. Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

Storage and stability
Store at 2 - 8 °C away from direct light. When stored as described above, reagents are stable until the expiry date stated on the label. A slight variation in the composition of the reagents can occur from batch to batch, without affecting the test results. After opening, they are stable for 30 days if closed immediately and protected from contamination, evaporation, direct light and stored at the correct temperature.

SAMPLE COLLECTION
Type of sample and storage
Fresh non-haemolysed serum or heparinised plasma samples should be used. GPT is stable in serum or plasma for 7 days at 4 - 8°C and 12 months at - 20°C (3) .

Precautions
All human samples must be handled and disposed of as potentially infectious materials.

PROCEDURE
Quality control
Control sera with a known titer of ALT are commercially available for quality control, including certificates of analysis showing values and confidence limits. Normal and abnormal control sera are available as "Normal control serum" code R0400000006 and "Pathological control serum" code R0400000106. The values obtained must be contained within the acceptability range. In case of incorrect results check the following points:
- Cleanliness of glassware.
- Wavelength setting.
- Expiration date of reagents.

Automation
Although this device has been developed and manufactured to be used by manual method and instrumental systems, it can also be used in combination with other instrumental devices that are able to meet the requirements indicated in the paragraph "Reaction conditions / Technique".
All applications not explicitly approved by. cannot be guaranteed in terms of performance and will therefore have to be evaluated by the user.

Calibration
For calibration use the kit "Multicalibrator" code R0300000006. For the use on ISE srl instruments Calibration every 10 days is recommended.

Traceability:
The ALT value is visible in the insert of the calibration serum package.

Reaction conditions

Wavelength (primary):	340 nm
Wavelength (secondary):	380 nm
Temperature:	37°C

Technique - Procedure with Reagent B as starter
Bring the reagents to the reaction temperature.

	U.M.	**Calib. Serum**	**Sample**
Reagent A	µL	1000	1000
Calib. Serum	µL	85	-
Sample	µL	-	85
Mix, after 2 minutes add:			
Reagent B	µL	100	100

Mix gently and incubate at 37°C. After incubation, add reagent B and read absorbance at 340 nm after 30 seconds. Repeat readings every 30 seconds or every 60 seconds. At least 3 repetitions of reading in the chosen times are recommended. Mean between Δ D.O./min.
The reaction volumes can be varied proportionately, the calculation remaining unchanged.

Results
The concentration of ALT-GPT is obtained through the following formula:

$$\frac{\Delta \text{ D.O. Simple}}{\Delta \text{ D.O. Calib. Serum}} \times \text{Calib.Serum. (U/L)} = \text{U/L di ALT-GPT}$$

Calculation of results obtained against multiplication factor

Δ D.O./min x K-factor* = U/L di ALT-GPT

Explanation of the formula:

$$\frac{Vt \times 1000}{C.M.E. \times P.O. \times Vc} K - Factor^* \times \Delta D.O./min. = U/L \text{ di ALT - GPT}$$

*K-factor (monoreagnt method) = 2090
*K-factor (bireagent method) = 1961

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where:
U/L = activities in international units per liter
Δ D.O./min. = change in absorbance per minute
Vt = total reaction volume (µL)
1000 = conversion of concentration per litre
C.M.E= ceffi. NADH extinction micromolar 6.22 cm2/mol at 340 nm
P.O= optical path (1.0 cm)
Vc = sample volume in the final reaction mixture (µL)

Reagents included in the kit
The reagents are described above.

Materials required but not supplied in the kit
Controls and calibrators.

NORMAL VALUES
Serum or Plasma:
- Male: < 40.0 U/L
- Female: < 35.0 U/L

Each laboratory must establish its own normal values on the basis of its local catchment area.

ANALYTICAL CHARACTERISTICS/PERFORMANCE
Linearity
The method is linear up to the following values of 400 U/L at 340 nm.

Specificity
The method is specific for the determination of ALT/GPT. Any eventual nonspecific reaction terminates within the delay period before taking the reading.

Accuracy – Recovery
The recovery of ALT/GPT added to a normal sample at known concentrations showed a result of 92.6%.

Interferences
Triglycerides below 2000 mg/dL does not interfere in the reaction. Ascorbic Acid influences the reaction at concentrations over 30 mg/dL.

Precision of the method

Within-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	24.8	2.284	9.21%	20
High	U/L	196	2.262	1.15%	20
Between-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	24.8	1.056	4.26%	20
High	U/L	196	7.886	4.02%	20

Sensitivity
At λ 340 nm a concentration of about 5.41 U/L of ALT/GPT in the conditions established for this test.

Comparative method
The method was compared with a similar method, as described in the IFCC optimization. Samples tested = no. 120; y intercept = 1.0877 + 2.49; Correlation Coefficient r = 0.9815.

Disposal of reagents
Disposal of reagents must be performed in accordance with the EC regulations regarding waste, or the local national or regional legislation.

Manufacturer:
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Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

Reference

1. Recommendation on I.F.C.C. methods for measurement of catalytic concentrations of enzymes, Clin Chem, 23:5 (1977).
2. Wroblewsky F., Ladue J.S., Proc. Soc. Exper. Biol and Med, 91:569 (1965).
3. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
4. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.

REVISION	DATE	CHANGE
Rev.A	12/2022	New Issue for IVDR Regulation (UE) 2017/746 compliance