ISE S.r.l.
CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

ALT - GPT – Instructions for use

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

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 picture.

INTRODUCTION
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

REAGENTS
Kit 4 x 50 mL code A-R0200001001
Reagent 1: no. 4 vials x 45.5 mL enzymatic reagent code 184012
Reagent 2: no. 4 vials x 5.0 mL NADH solution code 184013

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
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 directive 1272/2008 and subsequent amendments and additions. However, It is recommended to handle reagents according to the standards of good laboratory practice

Storage and stability
Store at 2 - 8°C and protect from direct light. When correctly stored, the reagent is stable up to the expiry date reported on the label.
A slight variation in the composition of the components may occur between batches, but this has no effect on the test results.
After opening, the vial R1 and R2 are stable 30 days if recapped immediately and protected from contamination, evaporation, direct light and stored at 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
Human control serum with known levels of ALT is commercially available for quality control purposes. Data sheets are included, listing the values and the confidence limits. Normal and abnormal control sera are available from I.S.E. S.r.l. "Normal control serum" code R0400000006 and "Pathological control serum" code R0400000106. Obtained values must be within the range of acceptability. If erratic results occur, the following points should be checked:
- Cleanliness of glassware.
- Wavelength setting.
- Expiration date of reagents.

Automation
This kit, though developed and manufactured to be used as manual assay and with I.S.E. S.r.l. analyzer, can be used also with other analyzers able to meet the specifications indicated in section "Reaction conditions - Test procedure" Application sheets are available for automatic instruments.
All applications not explicitly approved by I.S.E. S.r.l. cannot be guaranteed in terms of performance, and must therefore be established by the operator.

Calibration
For calibration use the "Multicalibrator" I.S.E. S.r.l. code R0300000006.

Traceability:
The ALT value is reported in the package insert supplied with the "Multicalibrator".

Calibration Stability
For the instrumentation series Miura, the calibration is recommended to be done every 10 days.

Method for automated instrumentation

Analyzer:	**I.S.E. Miura**		
Analyte Name :	**ALT GPT**	Ref.:	A-R0200001001
Method Code:	**GPT**		
Type:	**Kinetic**		
Unit:	**UI/l**		
Filter F1:	**340 nm**		
Blank in:	**Not Used**		
Step	**Reaction volume**		**U.M.**
Volume reagent R1:	**180**		µL
Volume reagent R2:	**18**		µL
Sample volume:	**20**		µL
Final Incubation:	**120**		Sec.
Kinetic reading time	**240**		Sec.

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 non specific reaction terminates within the delay period before taking the reading.

I.S.E S.r.l.
Via Delle Driadi, 45 – 00133 Roma
Tel.+39 077 4579365; FAX +39 077 4579305
E-mail: info@logotech-ise.com
www.logotech-ise.com

Revision F A-R0200001001-02-2016

ISE S.r.l.
CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

ALT - GPT – Instructions for use

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

IVD CE

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 I.S.E. S.r.l. ALT/GPT 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.

The product is in conformity with D.L: 8 September 2000, no. 332 "Actuation of the directive 98/79/EC regarding in vitro medical diagnostic devices".

Symbols used on labels and packaging

IVD = In vitro diagnostic medical device
REF = Catalogue Number
LOT = Lot Number
= Manufacturer
= Expiration date
= Temperature limitation
= Instruction for use

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.

I.S.E S.r.l.
Via Delle Driadi, 45 – 00133 Roma
Tel.+39 077 4579365; FAX +39 077 4579305
E-mail: info@logotech-ise.com
www.logotech-ise.com