ALT - GPT – method Kinetic Enzymatic
Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182521	n° 7 vials x 45 mL (R.A) n° 7 vials x 5 mL (R.B)
	REF B75182522	n° 8 vials x 18 mL (R.A) n° 8 vials x 2 mL (R.B)
CHEMILAB	REF B81180261	n° 4 vials x 34 mL (R.A) n° 1 vials x 13 mL (R.B)
	REF B81180262	n° 8 vials x 34 mL (R.A) n° 2 vials x 13 mL (R.B)

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 OF THE METHOD
Method Kinetic Enzymatic. 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.

Storage and stability

= storage temperature 2-8°C
stored at 2-8 ° C avoiding direct light, the reagents are stable until the expiration date printed on the label.

Concentrations

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

* Warning: The product is not classified, according to CLP

Reagents included in the kit
The reagent is described above.

Materials required but not supplied in the kit
Controls, calibrators and pipettes with adequate volume.

PRECAUTIONS and WARNINGS
1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2.Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting of serious incidents
Please inform the manufacturer (through your distributor) and the competent authority of the member state of the European Union in which the user and/or patient is established, of cases of serious incident that has occurred in relation to the device. For other jurisdictions, reports of serious incidents must be made in accordance with the regulatory requirements of the home Member State. By reporting serious incidents, you help provide more information about the safety of your in vitro medical diagnostic device.

PROCEDURE
Quality control
Control sera with a known titer of ALT-GPT are commercially available for quality control, with values and confidence limits included. Sclavo Diagnostics Normal and pathological control sera are available: Clinicontrol N 5x5mL cod. B35181700 and Clinicontrol A 5x5 mL code B35181701. The values obtained must be within the acceptability range.

Calibration
For calibration use the "Calibrator serum Sclavo" Code B35181702.

Traceability
The ALT-GPT traceability is reported in the package insert supplied with the calibrator serum.

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

REAGENT PREPARATION
The reagent is liquid, ready for use. After opening, the reagent is stable for 30 days if closed and stored at 2-8°C protected from direct light. Do not mix different batches. Slight variations in colour from batch to batch, will not affect test results.
- **Preparation of the reagent (Bireactive method)**
  Reagent A and Reagent B ready for use. After opening the stability of the reagents is 30 days if closed and stored at 2-8°C.
- **Preparation of the reagent (Monoreactive method)**
  Add 1 volume of Reagent vial B to 9 volume of reagent A, and mix by gently. After mixing the reagent is stable for 15 days if closed and stored at 2-8°C.

Automation
The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R1 / R2 / C. Validated applications are available for Sclavo Konelab® - Indiko® and CHEMILAB instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

MANUAL METHOD
The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

Reaction conditions:

Wavelength (primary):	340 nm
Temperature:	37°C.
Reaction	Kinetic (decreasing reaction)

Technique – Monoreactive procedure
Bring the reagents to reaction temperature and operate away from direct light.

	U.M.	Calibrator serum	Sample
Reagent	µL	1000	1000
Calibrator serum	µL	85	-
Sample	µL	-	85

Technique – Bireactive procedure

	U.M.	Calibrator serum	Sample
Reagent A	µL	1000	1000
Calibrator serum	µL	85	-
Sample	µL	-	85
Wait for 2 min.			
Reagent B	µL	100	100

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Mix gently and incubate at reaction temperature (37°C) for 2 minutes. After the incubation, read the absorbance at 340 nm. Repeat readings at 30 seconds or 1-minute intervals. Recording a minimum of 3 absorbance changes is recommended. Determine the mean ΔO.D./min.
The reaction volumes may be varied proportionally without alteration of results.

Results:
Manual Method
Calculation of ALT-GPT:

$$\frac{\Delta \text{D.O. Sample}}{\Delta \text{D.O. Calibrator Serum}} \times \text{Conc. Calibrator Serum} = \text{ALT - GPT U/L}$$

Automation
The results are automatically calculated by the analyzer based on the calibration line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration line is calculated automatically by the different instruments.
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Calculation of the results obtained using the multiplication factor
ΔO.D./min x K-factor* = U/L of ALT/GPT
Explanation of the calculation:

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

*K-factor (mono-reagent method) = 2090
*K-factor (two-reagent method) = 1961
where:
U/L = activity of the serum in international units
ΔO.D./min = variation in the absorbance per minute
Vt = total reaction volume (μl)
1000 = conversion to the concentration per litre
M.E.C. = micromolar extinction coefficient of NADH 6.22 $cm^2/\mu mol$ at 340 nm
P.O. = Optic path (1 cm)
Vc = sample volume in the mixture (μl)

REFERENCE RANGE
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 population.

ANALYTICAL CHARACTERISTICS/PERFORMANCE
Linearity
The method is linear up to the following values:
Mono-reagent method: ΔO.D./min of - 0.204 equal to about 426 U/L at 340 nm.
Two-reagent method: ΔO.D./min of - 0.196 equal to about 384 U/L at 340 nm.

Recovery
Commercial control sera were analyzed with the ALT-GPT kit following the guidelines of the CLSI protocol. The data obtained are shown in the table below.

Range	Replicates	Mean	DS	CV%	Recovery
Low	5	31,8	0,44	1,41	106,4 %
High	5	240	2,07	0,86	114,5 %

Interferences

Interference	Limits
Triglycerides	**2000 mg/dL**
Ascorbic acid	**30 mg/dL**

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Mean	S.D.	C.V. (%)	N°
Low	U/L	31,6	0,79	2,23	30
High	U/L	88,7	1,65	1,86	30
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	N°
Low	U/L	31,7	0,53	1,68	20
High	U/L	85,5	1,98	1,41	20

Limit of Sensitivity
The Sensitivity limit has been measured using serial dilutions of high concentrated sera. Under the conditions established for this test the lowest detectable concentration is 4.0 U/L ALT/GPT.

Comparison between methods
The proposed method was compared with another commercially available method following the guidelines of the CLSI analyzing 200 human sera. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	-3.393
Slope	0.984
Correlation Coeff. (R)	0.998

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	

REFERENCES

1. **Thomas L.** Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). In: Thomas L, editor. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 55-65
2. **H. U. Bergmeyer, G. N. Bowers, Jr., M. Hørder, and D. W. Moss** (1977) Provisional Recommendations on I.F.C.C. methods for measurement of catalytic concentrations of enzymes, Clin Chem, 23:5; 887-899.
3. **Wroblewsky F., Ladue J.S.,** (1965). Proc. Soc. Exper. Biol and Med, 91:569
4. **NCCLS Document,** "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
5. **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.
6. **Clinical Laboratory Standards Institute (CLSI). User Verification of Performance for Precision and Trueness**; Approved Guideline – Second Edition. EP15-A2.
7. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guideline – Second Edition. EP05-A2.
8. **Clinical Laboratory Standards Institute (CLSI).** Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition. EP09-A3.
9. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition – EP17
10. **Clinical Laboratory Standards Institute (CLSI).** Interference Testing in Clinical Chemistry, – Third Edition. - EP07.
11. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Linearity of Quantitative Measurement Procedures, 2nd Edition - EP06.

REVISION	DATE	CHANGE
Rev.A	01/2023	New Issue for IVDR Regulation (UE) 2017/746 compliance

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