AST - GOT – method Kinetic Enzymatic
Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182523	n° 7 vials x 45 mL (R.A) n° 7 vials x 5 mL (R.B)
	REF B75182526	n° 8 vials x 18 mL (R.A) n° 8 vials x 2 mL (R.B)
CHEMILAB	REF B81180251	n° 4 vials x 34 mL (R.A) n° 1 vials x 13 mL (R.B)
	REF B81180252	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 GOT 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
Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) belong to the group of aminotransferases or transaminases; these catalyze the reversible transformation of α-keto-acids into aminoacids through the transfer of amino groups. AST and ALT are present in human plasma, bile, cerebrospinal fluid and saliva. The serum ALT and AST levels are increased in viral hepatitis and in other forms of hepatic disease. In spite of the fact that, in such cases, both enzymes show raised serum levels, ALT is the more specific enzyme for the diagnosis of hepatic damage. AST levels can rise in connection with cardiac or skeletal muscle damage, in addition to hepatic parenchymal tissue damage.

PRINCIPLE OF THE METHOD
Method Kinetic Enzymatic. In the presence of 2-oxoglutarate, aspartate is transformed into oxalacetate and glutamate by the aspartate aminotransferase (AST/GPT) present in the sample. In the presence of NADH and malate-dehydrogenase (MDH), oxalacetate is transformed into malate and NAD. The consumption of NADH over a given period of time, determined at λ 340 nm, is proportional to the GOT concentration in the test sample.

$$L-Aspartate + 2-Oxglutarate \xleftrightarrow{AST} L-Glutamate + Oxalacetate$$

$$Oxalacetate + NADH + H^{+} \xleftrightarrow{MDH} D-Malate + NAD^{+}$$

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 8.1 ± 0.2	88.0	mmol/L
L-Aspartate	265	mmol/L
MDH	≥ 462	U/L
LDH	≥ 660	U/L
2-Oxoglutarate	13.2	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

The reagent is not classified according to CLP Regulation

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 AST-GOT 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 AST/GOT traceability is reported in the package insert supplied with the calibrator serum.

SAMPLE
Type of sample and storage
Fresh non-hemolyzed serum or heparinized plasma samples should be used. GOT 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 is30 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 (A + B)	µL	1000	1000
Calibrator serum	µL	85	-
Sample	µL	–	85

Technique – Bireactive procedure

	U.M.	Sample	Calibrator serum
Reagent A	µL	1000	1000
Sample	µL	85	-
Calibrator serum	µL	-	85
Mix, incubate for for 2 min and then add:			
Reagent B	µL	100	100

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Mix gently and incubate at reaction temperature for 2 min.
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 result.

Results:
Manual Method
Calculation of AST-GOT concentration:

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

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.

Calculation of the results obtained using the multiplication factor
ΔO.D./min x K-factor* = U/L of AST/GOT
Explanation of the calculation:

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

*K-factor (monoreagent 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: < 37.0 U/L
- Female: < 31.0 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 - 0.213 equal to about 378 U/L at 340 nm.
Two-reagent method: ΔO.D./min of - 0.194 equal to about 380 U/L at 340 nm.

Recovery
Commercial control sera were analyzed with the AST-GOT 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	42.4	0.548	1.29	98.6 %
High	5	276	1.517	0.55	97 %

Interferences

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

Precision of the method

Within-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	36.7	1.00	2.72	30
High	U/L	190	3.45	1.80	30
Between-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	36.6	0.57	1.56	20
High	U/L	186	3.60	1.94	20

Limit of Sensitivity
The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is 4.0 U/L of AST/GOT.

Comparison between methods
The 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	-0,840
Slope	0.984
Correlation Coeff. (R)	0.994

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

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

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