GOT / AST

Kinetic method UV - IFCC

R1: 4 x 40 ml + R2: 4 x 10 ml — CL37-200
R1: 3 x 100 ml + R2: 1 x 75 ml — CL37-375

INTENDED USE

Kit for quantitative determination of Aspartate Amino Transaminase AST/GOT (EC 2.6.1.1.) in serum and plasma according to IFCC recommendations.

CLINICAL MEANING

The enzyme AST is localysed in cellular cytoplasm and it is involved in amino acids metabolism. This enzyme is released in blood after the damage/death of cells: after a damage, AST reaches its maximum level in around 12 hours and it decreases in 4-5 days. The test is commonly used in association with the other transaminase (ALT) for the research of hepatic damage of any cause.

PRINCIPLE

In presence of α-ketoglutarate, AST/GOT in the sample transforms aspartate into oxalacetate and glutamate. In presence of NADH and malate dehydrogenase, oxalacetate is converted into malate and NAD. NADH oxidation in time unit at 340 nm is proportional to the AST/GOT concentration in the sample.

SAMPLE

Serum, EDTA or heparinized plasma. Avoid hemolyzed samples.
AST/GOT activity in serum decreases after 3 days at 2-8°C.

REAGENTS

Only for in Vitro diagnostics. Liquid reagents ready to use. Dangerous reagents are marked by an asterisk *

Package content	CL37-200	CL37-375
REAGENT 1 Tris buffer (pH 8,1) 88 mmol/L, L-asparate 265 mmo/L, MDH ≥ 462 U/L, LDH ≥ 660 U/L, α-ketoglutarate 13,2 mmol/L, sodium azide 30 mmol/L	4 x 40 ml	3 x 100 ml
REAGENT 2 Tris buffer (pH 10.2) 10 mmol/L, NADH 2,6 mmol/L, sodium azide 30 mmol/L	4 x 10 ml	1 x 75 ml

Stability: reagents are ready to use. Store at 2-8°C and protect from light to keep the reagents stable up to the expiration date on the label. Once opened reagents are stable for 2 months at 2-8°C if contamination is avoided. Keep bottles closed when not in use. Do not use turbid reagents.

NECESSARY ITEMS – NOT PROVIDED

Usual laboratory equipment: UV/VIS Spectrophotometer with temperature control; automatic micropipettes; Optical glass cuvettes or, alternatively, disposable ones in optical polystyrene; Saline solution.

REAGENT PREPARATION (for mono-reagent procedure only)

Mix 10 volumes of Reagent 1 with 1 volume of Reagent 2.
Stability: 5 days at 20-25°C or 4 weeks at 2-8°C if stored in a closed bottle protected from light.

MANUAL ASSAY PROCEDURE

Method:	decreasing kinetic
Wavelength:	340 nm
Optical path:	1 cm
Temperature:	30 o 37°C
Reading time:	3 minutes
Reading:	against air or distilled water
Sample/reagent Ratio (bireagent):	1/8/2
Sample/reagent Ratio (monoreagent):	1/10

BIREAGENT PROCEDURE

Bring reagents to the chosen temperature for the analysis.

Pipette in cuvette:

Sample	125 µl
Reagent 1	1,0 ml

Mix and incubate 1 minute at the chosen temperature. Add:

Reagent 2	250 µl

Mix and pour into the test cuvette. Incubate at the test temperature for 1 minute. Read initial absorbance, repeat reading at constant intervals of 1 minute for 3 minutes. Calculate the average value of the absorbance variations per minute (ΔA/min).

Reaction volumes can be proportionally varied without any change in calculation.

MONOREAGENT PROCEDURE

Let the working reagent necessary to perform the test reach the chosen temperature for the analysis.

Pipette in cuvette:

Sample	100 µl
Working reagent	1,0 ml

Mix and pour into the test cuvette. Incubate at the test temperature for 1 minute. Read initial absorbance, repeat reading at constant intervals of 1 minute for 3 minutes. Calculate the average value of the absorbance variations per minute (ΔA/min).

Reaction volumes can be proportionally varied without any change in calculation.

CALCULATION

To calculate the enzymatic activity in the sample, multiply ΔA/min by the proper factor from the following table.

Activity in U/l: ΔA/min x 1746

Activity in µkat/l: U/l x 0.0167

REFERENCE VALUES

	30°C	37°C
Men	up to 25 U/L	up to 37 U/L
Women	up to 21 U/L	up to 31 U/L

Each laboratory should define its own reference values for this method.

QUALITY CONTROL – CALIBRATION

All Clinical Chemistry laboratories should implement a quality control program. Control serums of human origin are available for this purpose on request:
PRE-NORM serums with normal values
PRE-PATH serums with pathological values
If the method requires it, a multiparameter calibrator of human origin is available.

PERFORMANCE CHARACTERISTICS

Sensitivity: the sensitivity of the method is 3 U/L.

Linearity: up to 300 U/L.
For higher values, dilute the sample 1:10 with saline solution and multiply the result by 10.

Precision:

Within run (n=10)	Average [U/L]	SD	CV %
Sample 1	23,5	0,95	4
Sample 2	238	3,36	1,4

Between run (n=20)	Average [U/L]	SD	CV %
Sample 1	27	1,41	4,2
Sample 2	222	7,05	3,17

Interferences: lipids up to 2000 mg/dl of triglycerides do not interfere. Up to 40 mg/dl of bilirubin does not interfere. Up to 30 mg/dl of ascorbic acid does not interfere. Hemolysis presence in the sample may give falsely positive values.

Correlation against a reference method: the correlation of FAR method (Y) against a reference method (X) gives this equation:

Y = 1,0681X - 3,0802 r = 0,9712

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

REAGENT 1 and REAGENT 2 **WARNING:** ***H412*** is toxic for aquatic organisms with long term effects. ***H319*** *causes severe eye irritation.* ***H315*** Causes skin irritation.

REFERENCES

1. Recommendation on I.F.C.C. methods for measurement of catalytic concentrations of enzymes, Clin Chem, 23:5 (1977) Wroblewsky F., Ladue J.S., Proc. Soc. Exper. Biol and Med, 91:569 (1965)
2. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).

MANUFACTURER

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalog number
	temperature limits
	use by
	attenzione
	consult accompanying documents

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