ALT / GPT

Kinetic method UV - IFCC

R1: 5 x 50 ml + R2: 1 x 25 ml CL38-275

INTENDED USE

Kit for quantitative determination of Alanine Amino Transaminase ALT/GPT (EC 2.6.1.2.) in serum and plasma according to IFCC recommendations.

CLINICAL MEANING

Alanine Amino Transaminase (ALT) is found mainly in liver and kidney cells. Its function is to convert alanine into pyruvate, a chemical compound, important for the production of cellular energy. In healthy individuals, ALT levels are low and they increase when the liver is damaged. The analysis is therefore very useful for the early diagnosis of hepatic diseases.

PRINCIPLE

In presence of α-ketoglutarate, alanine is transformed into pyruvate and glutamate by ALT/GPT in the sample. In presence of NADH and lactate dehydrogenase, pyruvate is converted into lactate and NAD.
NADH oxidation in time unit at 340 nm is proportional to the ALT/GPT concentration in the sample.

SAMPLE

Serum (preferably), plasma (not recommended). Avoid hemolyzed samples.
STABILITY: 3 days at 2-8°C, 1 month at 20°C.

REAGENTS

Only for in Vitro diagnostics. Liquid reagents ready to use.

Package contents	CL38-275
REAGENT 1 Tris buffer (pH 7,8) 110 mmol/L, L-alanine 550 mmo/L, LDH ≥ 1320 U/L, sodium azide 30 mmol/L , α-ketoglutarate 16,5 mmol/L,	5 x 50 ml
REAGENT 2 Tris buffer (pH 10.2) 10 mmol/L, NADH 2,6 mmol/L, sodium azide 30 mmol/L	1 x 25 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.

PREPARATION OF WORKING REAGENT (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 (334 - 365)
Optical path:	1 cm
Temperature:	30 or 37°C
Rate Time:	3 minutes
Measurement:	against air or distilled water
Sample/Reagents (bi-reagents):	1/10/1
Sample/Reagent (mono-reagent):	1/10

Bi-reagent procedure

Bring the necessary reagents to the chosen temperature for the analysis.
Pipette in cuvette:

Sample	100 µl
Reagent 1	1,0 ml

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

Reagent 2	100 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).

Mono-reagent procedure

Bring the necessary reagents to 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

CALCULATION

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

λ	Mono reagent Procedure	Bi reagent Procedure
334 nm :	1780	1945
340 nm :	1746	1905
365 nm :	3235	3529

REFERENCE VALUES

	30°C	37°C
Men	up to 25 U/L	up to 40 U/L
Women	up to 22 U/L	up to 35 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	26,8	230	3,6
Sample 2	0,97	2,96	1,28

Between run (n=20)	Average [U/L]	SD	CV %
Sample 1	26,8	215	3,6
Sample 2	0,97	7,05	3,28

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 the method (Y) against a reference method (X) gives this equation:

Y = 1,0356X + 0,4362 r = 0,9975

DISPOSAL

P501: dispose of the product according to national legislation.

WARNINGS AND PRECAUTIONS

REAGENT 1 and REAGENT 2 **WARNING:** ***H412*** Harmful 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)
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

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KEY SYMBOLS

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

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