ISE S.r.l. ALT/GPT – Instructions for use

CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

CE IVD REF

R1: 2x 18 mL R2: 2x 4,5 mL **R3330000008**

Diagnostic reagent for quantitative in vitro determination of GPT (ALT) in human serum or plasma on photometric systems.

TEST PARAMETERS

Method:	UV, Kinetic, Decreasing Reaction, modified IFCC
Wavelength:	340 nm, Hg 334 nm, Hg 365 nm
Temperature:	37°C
Sample:	Serum, EDTA-plasma, heparinized plasma
Linearity:	up to 600 U/L
Sensitivity:	The lower limit of detection is 4 U/L.

SUMMARY [1,2]

Alanine Aminotransferase (ALAT/ALT), also called Glutamic Pyruvic Transaminase (GPT) and Aspartate Aminotransferase (ASAT/AST), formerly called Glutamic Oxalacetic Transaminase (GOT) are the most important representatives of a group of enzymes, the aminotransferases or transaminases, which catalyse the conversion of a-keto acids into amino acids by transfer of amino groups.
As liver specific enzyme GPT is only significantly elevated in hepatobiliary diseases, Increased GOT levels, however, can occur in connection with damages of heart or skeletal muscle as well as of liver parenchyma. Parallel measurement of GPT and GOT is therefore applied to distinguish liver from heart or skeletal muscle damages. The GOT/GPT ratio is used for differential diagnosis in liver diseases. While ratios < 1 indicate mild liver damage, ratios > 1 are associated with severe, often chronic liver diseases.

TEST PRINCIPLE

L-Alanine + 2-Oxoglutarate $\xrightarrow{GPT}$ Pyruvate + L-Glutamate

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ L-Lactate + NAD^+

NADH is oxidized to NAD^+, the resulting decrease in absorbance at 340 nm is directly proportional to the activity of GPT in the sample.
This is a modified formulation for the assay of GPT, as recommended by the IFCC (International Federation of Clinical Chemistry). The IFCC reference method includes pyridoxal phosphate (P-5-P). P-5-P serves as coenzyme in AA transfer and stabilizes the activity of transaminases. Therefore addition of P-5-P avoids falsely low values in samples containing insufficient endogenous P-5-P, e.g. from patients with myocardial infarction, liver disease and intensive care patients [1].

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
Reagent 1:		
Tris, pH 7.5	138	mmol/L
L-Alanine	709	mmol/L
LDH	1500	U/L
Reagent 2:		
2-Oxoglutarate	85	mmol/L
NADH	≥ 1	mmol/L

ABBREVATIONS

AA	=	Amino Acid
GPT	=	Glutamate Pyruvate Transaminase
NAD^+	=	Nicotinamide Adenine Dinucleotide
NADH	=	reduced NAD
LDH	=	Lactate Dehydrogenase

REAGENT PREPARATION

Substrate Start:
Reagents are ready for use.
Sample Start:
Mix 4 parts of Reagent 1 with 1 part of Reagent 2.
(= Working Reagent)

REAGENT STABILITY AND STORAGE

Conditions: protect from light. Avoid contamination. Close immediately after use. Do not freeze the reagents!

Substrate Start:

Stability: at 2 – 8°C up to the expiration date

Sample Start (Working Reagent):

Stability: at 2 – 8 °C 4 weeks
at 15 –25 °C 5 days

The working reagent must be protected from light!

SAMPLE STABILITY AND STORAGE

Stability [4]: at 20 – 25 °C 3 days
at 4 – 8 °C 7 days
at - 20 °C 7 days

Discard contaminated specimens. Freeze only once!

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment
Pyridoxal-5' Phosphate in case of determination with P-5-P-

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.
Note: If pyridoxal phosphate (PP) is used, please consult instruction insert for PP before performing test (for Substrate Start only).

Substrate Start

Pipette into test tubes	37°C
Reagent 1	1000 µl
Sample	100 µl
Mix. Incubate for 5 min. Then add:	
Reagent 2	250 µl
Mix. Read initial absorbance against air after 1 min. and start a timer. Read absorbance again after exactly 1, 2 and 3 min.	

Sample Start (do not use sample start with P-5-P)

Pipette into test tubes	37°C
Working reagent	1000 µl
Sample	100 µl
Mix. Read initial absorbance against air after 1 min. and start a timer. Read absorbance again after exactly 1, 2 and 3 min.	

CALCULATION

With factor (light path 1 cm)**:**

From absorbance readings calculate ΔA/min and multiply by the corresponding factor:
GPT [U/L] = ΔA/min x factor
Factors (37 °C):

Substrate Start:

Factor at 340 nm	2143
Factor at 334 nm	2184
Factor at 365 nm	3971

Sample Start:

Factor at 340 nm	1745
Factor at 334 nm	1780
Factor at 365 nm	3235

With calibrator:

$$\text{GPT [U/L]} = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \times \text{Conc. Calibrator [U/L]}$$

UNIT CONVERSION

U/L x 0.01667 = µkatal/L

REFERENCE RANGE*

IFCC, 37 °C:

		U/L	µkat/L
Women [3]		< 34	< 0.57
Men [3]		< 45	< 0.75
Children [1]	1 – 30 days	< 25	< 0.42
	2 – 12 months	< 35	< 0.58

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	1 – 3 years	< 30	< 0.50
	4 – 6 years	< 25	< 0.42
	7 – 9 years	< 25	< 0.42
	10 – 18 years	< 30	< 0.50

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

On automated systems the test is suitable for the determination of GPT activities up to 600 U/L.
In case of manual procedure, the test is suitable for GPT activities which correspond to a maximum ΔA/min = 0.16 at 340 nm and 334 nm or 0.08 at 365 nm.
Above this concentration the samples should be diluted 1+9 with NaCl solution (9 g/L) and the results multiplied by 10.

SENSITIVITY/LIMIT OF DETECTION
The lower limit of detection is 4 U/L
PRECISION **(at 37°C)**

Without P-5-P

Intra-assay, n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	22.2	1.38	6.22
Sample 2	44.8	1.17	2.62
Sample 3	101	1.02	1.00

Inter-assay, n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	22.8	0.70	3.08
Sample 2	42.6	0.68	1.60
Sample 3	99.3	0.92	0.92

With P-5-P

Intra-assay, n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	33.8	1.25	3.71
Sample 2	72.0	2.04	2.83
Sample 3	128	2.77	2.16

Inter-assay, n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	33.3	0.99	2.96
Sample 2	72.1	1.36	1.88
Sample 3	133	1.76	1.32

SPECIFICITY/INTERFERENCES

no interference up to:

ascorbic acid	30 mg/dl
bilirubin	40 mg/dl
hemoglobin	400 mg/dl
triglycerides	2000 mg/dl

For further information on interfering substances refer to Young DS [5].

METHOD COMPARISON

Without P-5-P

A comparison between this GPT (ALT) without P-5-P (y) and a commercially available test (x) using 105 samples gave following results: y =1.024 x – 1.199 U/L; r = 0.999.

With P-5-P

A comparison between this GPT (ALT) with P-5-P (y) and a commercially available test (x) using 107 samples gave following results: y = 1.027 x – 0.189 U/L; r = 1.000.

CALIBRATION

The use of a GPT Calibrator is optional. We recommend our Multicalibrator which has been standardized against the original IFCC formulation.

QUALITY CONTROL

All control sera with GPT values determined by this method can be used. We recommend our serum controls Normal control serum (control serum with values in the normal range) and Pathological control serum (control serum with values in the abnormal range). Each laboratory should establish corrective action in case of deviations in control recovery.

WARNINGS AND PRECAUTIONS

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. In very rare cases, samples of patients with gammopathy might give falsified results [6].
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
4. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

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5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
6. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.

Symbols on labels and packaging

IVD = In vitro diagnostic medical device

REF = Catalog Number

LOT = Lot Number

= Manufacturer

= Expiration date

= Temperature limitation

= Instruction for use

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