LIQUID LINE

GPT(ALT) UV AA

Optimized UV method (IFCC) for the determination of Alanine Aminotransferase (ALT/GPT) in serum or plasma

SUMMARY

Alanine Aminotransferase (ALT or GPT) is an unilocular (cytoplasmic) enzyme, and its highest activity is located in the hepatic tissue. The destruction or any change in the permeability of the cellular membranes causes the releasing of ALT into the blood stream.
The highest increases of ALT activity in serum are caused by hepatic alterations.
In the case of viral hepatitis, the increase of ALT precedes jaundice, reaching the maximum after this symptom is observed. If the values remain high after 6 weeks, the possibility of an active hepatitis or the beginning of a chronic hepatitis must be inferred, that is why the serial determinations of the enzyme are very useful.
ALT determination becomes of diagnostic importance when its values are compared with the values of other enzymes of similar tissue origin, allowing to complete the enzymatic profile of organs such as the liver.

PRINCIPLE

The reaction system is as follows:

$$\text{L-alanine} + \text{2-oxoglutarate} \xrightarrow{\text{GPT}} \text{pyruvate} + \text{L-glutamate}$$

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{l-lactate} + \text{NAD}^+$$

PROVIDED REAGENTS

A. Reagent A: TRIS buffer solution, pH 7.5 with L-Alanine.
B. Reagent B: vials containing 2-Oxoglutarate, reduced NADH and Lactate Dehydrogenase (LDH).

Final concentrations

TRIS	100 mmol/l; pH 7.5
L-Alanine	500 mmol/l
NADH	0.18 mmol/l
LDH	≥ 1.5 U/l
2-Oxoglutarate	15 mmol/l

INSTRUCTIONS FOR USE

Provided Reagents: ready to use. They may me used separately or as **Monoreagent**, mixing 4 parts Reagent A and 1 part Reagent B (e.g. 4 ml Reagent A + 1 ml Reagent B).

WARNINGS

Reagents are for “in vitro” diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10°C) until the expiration date shown on the box.
Once opened, do not keep out of the refrigerator for extended periods of time. Avoid contamination.
Monoreagent (premixed): stable in refrigerator for 2 months (2-10°C) from reconstitution date.

INSTABILITY OR DETERIORATION OF REAGENTS

When spectrophotometer has been set to zero with distilled water, absorbance readings of the Monoreagent < 0.900 O.D. or > 1.800 O.D. (at 340 nm), indicate its deterioration.

SAMPLE

Serum or plasma
a) Collection: obtain in the usual way.
b) Additives: when using plasma, only use heparin or EDTA as anticoagulants.
c) Known interfering substances:
- Samples from hemodialyzed patients or patients with hypovitaminosis of other pyridoxal phosphate deficiencies produce falsely decreased values.
- No interferences are observed by bilirubin up to 25 mg/dl, triglycerides up to 1000 mg/dl. Hemoglobin significantly interferes increasing the results because of GPT presence in erythrocytes.

See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: GPT in serum is stable in refrigerator up to 3 days without preservatives. Do not freeze.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer.
- Micropipettes and pipettes for measuring the stated volumes
- Water bath at temperature indicated under PROCEDURE.
- Stopwatch.

ASSAY CONDITIONS

- Wavelength: 340 nm
- Reaction temperature: 25, 30 or 37°C. See REFERENCE VALUES corresponding to each temperature.
- Reaction time: 4 minutes.
- Sample volume: 100 ul.

Sample and Reagent volumes may be proportionally changed, without altering the corresponding calculation factors.

PROCEDURE

A) 30 or 37°C

I- MONOREAGENT TECHNIQUE
In a cuvette at 30-37°C, place:

Monoreagent	1.0 ml

Preincubate for a few minutes. Then add:

Sample	100 ul

Mix immediately and simultaneously start stopwatch. After 90 seconds record initial absorbance (see PROCEDURE LIMITATIONS) and then at 1, 2 and 3 minutes after the first reading. Determine average change in Absorbance/min (ΔA/min), subtracting each reading from the previous one and averaging these values. Use this mean for calculations.

II- SEPARATE REAGENTS TECHNIQUE
In a cuvette at 30-37°C, place:

Reagent A	0.80 ml
Sample	100 ul

Preincubate for a few minutes. Then add:

Reagent B	0.20 ml

Mix immediately and simultaneously start stopwatch. After 90 seconds record initial absorbance (see PROCEDURE LIMITATIONS) and then at 1, 2 and 3 minutes after the first reading. Determine average change in Absorbance/min (ΔA/min), subtracting each reading from the previous one and averaging these values. Use this mean for calculations.

B) 25°C

Use 250 ul Sample and follow the procedure described in A).

CALCULATIONS

GPT (U/l) = ΔA/min x factor

In each case, the corresponding calculation factor should be used, depending on the selected reaction temperature:

factor $_{(30\text{-}37^{o}C)}$ = 1,746

factor $_{(25^{o}C)}$ = 794

REFERENCE VALUES

Temperature	25°C*	30°C*	37°C*
Men	up to 22 U/l	up to 29 U/l	up to 41 U/l
Women	up to 17 U/l	up to 22 U/l	up to 31 U/l

* Calculated

It is recommended that each laboratory establishes its own reference values.

SI SYSTEM UNITS CONVERSION

GPT (U/l) x 0.017 = GPT (ukat/l)

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known alanina aminotransferase activity.

PROCEDURE LIMITATIONS

See Known Interfering Substances under SAMPLE.

Low initial absorbance: when the first reading (0 time) is lower than 0.900 O.D. after the serum addition, being the Reagent B in good conditions, it indicates a sample with a very high GPT activity (that consumes NADH even before this reading) or with a particularly high concentration of endogenous ketoacids. In this case, repeat assay with the sample diluted with saline solution and multiply the results by the dilution performed.

PERFORMANCE

a) Reproducibility: testing replicates of the same sample simultaneously, the following values are obtained:

Level	S.D.	C.V.
43.35 U/l	± 1.31 U/l	3.02 %
119.70 U/l	± 2.18 U/l	1.82 %

b) Sensitivity: the smallest detectable GPT activity distingible from zero will be 2 U/l.

c) Dynamic range: the reading range is extended up to 0.345 ΔA/min (at 340 nm). If the ΔA/min is higher than 0.345, repeat the assay with diluted sample (1:5 or 1:10) with saline solution, correcting the results according to the dilution factor used.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user manual of the autoanalyzer in use.

WIENER LAB. PROVIDES

200 ml (Cat. Nº: 1762360): - 4 x 40 ml Reagent A
- 1 x 40 ml Reagent B

250 ml (Cat. Nº: 1009322): - 4 x 50 ml Reagent A
- 4 x 12.5 ml Reagent B

250 ml (Cat. Nº: 1009263): - 4 x 50 ml Reagent A
- 4 x 12.5 ml Reagent B

250 ml (Cat. Nº: 1009620): - 4 x 50 ml Reagent A
- 4 x 12.5 ml Reagent B

REFERENCES

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- SSCC - Scand. J. Clin. Lab. Invest. 33:291 (1974).
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Symbols

The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.

CE — This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Conteins sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Xn — Harmful

Corrosive / Caustic

Xi — Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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