γ-G-test
cinética AA

Modified Szasz method for the determination of γ-glutamyl transferase in serum or plasma. IFCC recommended substrate.

SUMMARY
γ-Glutamyl Transferase (γ-GT) is a membrane enzyme widely distributed in the body. It is primarily located in the kidney, seminal vesicles, pancreas, liver, spleen and brain. Its activity is influenced by any factor altering the cellular membranes of the organs that contain it. In the case of liver disorders, γ-GT generally indicates toxic aggression. However, its determination only has clinical relevance when its values are compared to those of other greater organ-specificity enzymes.
γ-GT determination together with alkaline phosphatase, transaminase and bilirubin, significantly broadens the spectrum for differential diagnosis of primary and secondary liver diseases, being part of the hepatic profile.

PRINCIPLE
γ-Glutamyl Transferase is a carboxypeptidase that catalyzes the following reaction:

$$\text{L-}\gamma\text{-Glutamyl-3-carboxy-4-nitroanilide + glycilglycine} \xrightarrow{\gamma\text{-GT}} \text{L-}\gamma\text{-Glutamyl glycilglycine + 5-amino-2-nitrobenzoate}$$

PROVIDED REAGENTS
A. Reagent A: vials containing L-g-Glutamyl-3-carboxy-4-nitroanilide and glycilglycine.
B. Reagent B: 100 mmol/l Tris buffer solution for final 8.25 pH at 25ºC.

Final concentrations

Tris buffer	100 mmol/l
L-g-Glutamyl-3-carboxy-4-nitroanilide	2.9 mmol/l
glycilglycine	100 mmol/l

INSTRUCTIONS FOR USE
Reagent B: ready to use.
Reagent A; reconsitution: reconstitute Reagent A with the amount of Reagent B stated on the label. Cap and shake until complete dissolution.

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: are stable in refrigerator (2-10ºC) until the expiration date shown on the box.
Reconstituted Reagent A: stable for 21 days in refrigerator (2-10ºC) and for 3 days at room temperature.

INSTABILITY OR DETERIORATION OF REAGENTS
Sediment and/or color change may indicate reagent deterioration.

SAMPLE
Serum or plasma
a) Collection: obtain sample in the usual way.
b) Additives: when using plasma, collect it with EDTA as anticoagulant.
c) Known interfering substances:
- Anticoagulants containing citrate, fluoride or oxalate produce a mild inhibition of enzyme activity, while heparin interferes.
- Sera with jaundice, moderate or strong hemolysis or hyperlipemia produce falsely increased values.

See Young, D.S. in References for effect of drugs on the present method.
d) Stability and Storage Instructions: γ-GT in serum is stable for 2 weeks in refrigerator (2-10ºC) and up to 6 months in freezer (-4ºC) without preservatives.

REQUIRED MATERIAL (non-provided)
- Spectrophotometer.
- Micropipettes and pipettes for measuring the stated volumes
- Spectrophotometric square cuvettes.
- Water bath at selected assay temperature.
- Stopwatch.

ASSAY CONDITIONS
- Wavelength: 405 nm
- Reaction temperature: 25, 30 or 37ºC
- Reaction time: 3 minutes
- Sample volume: 100 ul
- Reagent volume (Substrate): 1 ml
- Final reaction volume: 1.1 ml

PROCEDURE
In a cuvette kept at the selected temperature, place:

Reconstituted Reagent A	1 ml
Pre-incubate a few minutes. Then add:	
Sample	100 ul
Mix at once and continue incubation immediately. Adjust	

absorbances to a reference value (0.200 or 0.300 O.D.) and simultaneously start stopwatch. Record absorbance at 1, 2 and 3 minutes. Determine average absorbance change (ΔA/min) subtracting each reading from the previous one and averaging values. Use this mean for calculations.

CALCULATIONS

γ-Glutamyl Transferase (U/l) = ΔA/min x 1,158

SI SYSTEM UNITS CONVERSION

γ-GT (U/l) x 0.017 = γ-GT (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 γ-glutamyl transferase activity.

REFERENCE VALUES

Temperature	25ºC	30ºC*	37ºC*
Men	6-28 U/l	8-38 U/l	11-50 U/l
Women	4-18 U/l	5-25 U/l	7-32 U/l

*Calculated

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

Frequent values in various diseases

Pathology	Increase (nº of times) above higher reference limit
Hepatic Cirrhosis	1.5 to 15
Acute Hepatitis	2 to 20
Chronic Hepatitis	3 to 20
Fatty liver	up to 10
Extra hepatic obstruction with jaundice	1.5 to 20
Extra hepatic obstruction without jaundice	up to 20
Liver metastasis	up to 40
Chronic alcoholism	1.5 to 10

PROCEDURE LIMITATIONS

See Known Interfering Substances under SAMPLE.
Timing as well as reaction temperature are critical. For each degree of temperature variation, results increase or decrease approximately 5%.

PERFORMANCE

a) Reproducibility: when replicates of the same sample were assayed on the same day, the following results were obtained:

Level	S.D.	C.V.
69.9 U/l	± 1.13 U/l	1.62 %
188.6 U/l	± 1.34 U/l	0.71%

b) Detection Limit: it depends on the photometer used. According to the required sensitivity, in spectrophotometer at 405 nm (with 1 cm optical length square cuvettes, ± 2 nm reproducibility, ≤ 0.5% stray light, ≤ 8 nm pathlength) for a ΔA/min of 0.001 the minimum change of visible activity will be of 1.2 U/l.

c) Linearity: reaction is linear up to 250 U/l. For higher values, dilute sample 1/5 or 1/10 with saline solution and repeat assay under the same assay conditions. Multiply results by the dilution performed.

PARAMETERS FOR AUTOANALYZERS

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

WIENER LAB. PROVIDES

- 3 x 20 ml (60 ml Reagent B) (Cat. Nº 1421402).
- 20 x 3 ml (60 ml Reagent B) (Cat. Nº 1421403).

REFERENCES

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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 — Contains 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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