LIQUID LINE

γ-G-test
cinética AA

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

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:

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

PROVIDED REAGENTS
A. Reagent A: Tris buffer solution containing glycilglycine.
B. Reagent B: L-γ-Glutamyl-3-carboxy-4-nitroanilide solution.

Final concentrations

Tris buffer	100 mmol/l; pH 8.5
L-γ-Glutamyl-3-carboxy-4-nitroanilide	> 2.9 mmol/l
glycilglycine	100 mmol/l

NON-PROVIDED REAGENTS
Saline solution (9 g/l NaCl).

INSTRUCTIONS FOR USE
Provided Reagents: ready to use. They may be used separately or as **Monoreagent**, mixing 4 parts Reagent A with 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: are stable at 2-10ºC until the expiration date stated on the box.

Monoreagent (premixed)**:** stable for 4 weeks at 2-10ºC. Protect from sunlight.

INSTABILITY OR DETERIORATION OF REAGENTS
When the spectrophotometer has been set to zero with distilled water, absorbance readings of the Monoreagent higher than 1.300 O.D. 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: no interferences have been observed by bilirubin up to 280 mg/l (28 mg/dl), triglycerides up to 5.4 g/l (540 mg/dl) nor hemoglobin up to 0.39 g/dl (390 mg/dl). 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 at 2-10ºC and up to 6 months at -20ºC.

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 B volume (Substrate): 1 ml
- Final reaction volume: 1.1 ml

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

Monoreagent	1 ml
Pre-incubate a few minutes. Then add:	
Sample	100 ul

Mix at once and continue incubation immediately 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 running, 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

PROCEDURE LIMITATIONS
See Known Interfering Substances under SAMPLE.

PERFORMANCE
a) Reproducibility: CLSI protocol EP15-A was applied. Two activity levels were tested, in replicates by four, during 5 days. With the obtained data, total and intra-assay precision were calculated.

Intra-assay precision (n = 20)

Level	S.D.	C.V.
37.0 U/l	± 0.45 U/l	1.21 %
160.8 U/l	± 0.90 U/l	0.56 %

Total precision (n = 20)

Level	S.D.	C.V.
37.0 U/l	± 1.10 U/l	2.98 %
160.8 U/l	± 3.69 U/l	2.29 %

b) Linearity: manually, the reaction is linear up to 0.200 O.D. ΔA/min (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. In autoanalyzers, a linearity up to 1200 U/l may be observed.
c) Analytical sensitivity: depends on the photometer used and wavelength. In spectrophotometer with 1 cm optical length square cuvettes, for ΔA/min of 0.001, the minimum detectable activity change will be of 1 U/l.

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

WIENER LAB. PROVIDES
100 ml: - 4 x 20 ml Reagent A
- 1 x 20 ml Reagent B
(Cat. 1421404)

100 ml: - 4 x 20 ml Reagent A
- 1 x 20 ml Reagent B
(Cód. 1009330)

100 ml: - 4 x 20 ml Reagent A
- 1 x 20 ml Reagent B
(Cód. 1009258)

125 ml: - 5 x 20 ml Reagent A
- 2 x 12,5 ml Reagent B
(Cód. 1009616)

REFERENCES

- Lum, G.; Gambino, S.R. - Clin. Chem. 18:358 (1972).
- Burrows, S.; Feldman, W.; McBride, F. - Am. J. Clin. Path. 64/3:311 (1975).
- Szasz, G. - Clin. Chem. 15/2:124 (1969).
- S.S.C.C. - Scand. J. Clin. Lab. Invest. 36:119 (1976).
- Szasz G.; Weinmann G.; Staler F.; Wahlefeld W.; Persijn J. - Z. Klin. Chem. Biochem. 12:229 (1974).
- I.F.C.C. - J. Clin Chem. Clin. Biochem. 21: 633 (1983).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 5th ed., 2000.
- CLSI: Clinical and Laboratory Standards Institute (ex-NCCLS) - Protocols EP-15A, 2001 / EP-17A, 2004.

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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