GAMMA GT Liquiform
Insert

Ref.: 105

Intended use . Kinetic System for quantitative determination of Gamma - Glutamyltransferase (GAMA GT) in serum or plasma (EDTA).

Professional use.

[For in vitro diagnostic use]

Test principle . Gamma-Glutamyltransferase catalyzes the transferece of L-γ-Glutamyl-3-carboxi-4-nitroanilide to glycylglycine, yielding L-γ-Glutamylglycylglycine and p-nitroaniline as following the reactions:

$$\text{L-}\gamma\text{-Glutamyl-3-carboxi-4-nitroanilide} + \text{glycylglycine} \xrightarrow{\text{Gamma GT}} \text{L-}\gamma\text{-Glutamylglycylglycine} + \text{p-nitroaniline}$$

The amount of p-nitroaniline produced, which has high absorbance at 405 nm, is proportional to the Gamma GT activity in the sample.

Summary . Labtest developed Gamma GT Liquiform system based on the principle of the method of modified Szasz, that propitiates procedure for Gamma GT determination and whose performance is substantially equivalent to the reference method proposed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFFC)[1,2].

The substances for this reaction are distributed properly in two reagents in order to get more stability in the original liquid form and keep the optimum conditions of operation conditions, allowing the direct use of the reagents in automatic systems.

The monoreagent method can be applied by using one Work Reagent, 21 days stable if stored at 2-8ºC, reaching appropriated performance even in low requests of the test. The system also allows preparing the volume of the Work Reagent needed to one measure of the Gamma GT enzymatic activity.

The system is linear up to 700 U/L, equivalent to 15 times the high reference threshold, decreasing the dilution necessities and measurement repetitions in high activities sample.

The performed assays show that Labtest products do not suffer interference by high values of bilirubin, hemoglobin, and triglycerides.

The measurements can be done by the fixed-time-kinetic method, in semi-automatic equipments, using the p-nitroaniline Standard (Ref.: 105.3). Also the measurements can be done by the continuous kinetic method, in automatic and semi-automatic equipments. In this case, Labtest provides the Calibra's series with Gamma GT activity traceable to the reference material ERM®-AD452/IFCC of the Institute for Reference Materials and Measurements and to the reference method of the IFCC[2].

Methodology . Szasz modified.

Reagents

1. R 1 - Reagent 1 - Store at 2-8ºC.
Reagent label bears expiration date. Glycylglycine (197 mmol/L) and sodium azide (≤0.095%).

2. R 2 - Reagent 2 - Store at 2-8ºC.
Reagent label bears expiration date. L-γ-Glutamyl-3-carboxi-4-nitroanilide (21 mmol/L) and sodium azide (≤0.095%).

3. CAL - Standard - Store at 2-8ºC.
Reagent label bears expiration date. p-nitroaniline (500 μmol/L) and sodium azide (≤0.095%).
Do not contain gamma-glutamyltransferase.

The Standard is applicable only in the fixed-time-kinetic method, described in this inserts. The p-nitroaniline amount is equivalent to 125 U/L Gamma GT's activity.

Precautions and warnings

Avoid the directly sun light exposure.

Disposal of all waste material should be in accordance with local guidelines.

The usual security cares should be applied on the reagent handling.

The reagents contain sodium azide as preservative. Avoid ingestion. In case of eyes contact, immediately flush eyes with plenty of water and get medical assistance.
Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation.

In automatic equipments, the reagents may be contaminated with other reagents or the air, depending on the equipment's characteristic and the work conditions. These can result in stability reduction and calibration modifications.

As it occurs in all enzymatic activity measurement, the incubation time and temperature is important for the quality of the results.

Storage and stability . Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label.

Deterioration . Microbial or chemical contamination may decrease reagents stability.
Gamma GT is not suitable for use if Working Reagent has an absorbance over 1.5 at 405 nm when measured versus water as reference or in case of contaminations signs or if it develops turbidity.

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Sample

Use serum or plasma (EDTA). Gamma GT is reportedly stable for about 7 days at 2-8ºC and 2 months at -20ºC[3].

No known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious.

Interference

Bilirubin up to 38 mg/dL, hemoglobin up to 180 mg/dL and triglycerides up to 1000 mg/dL do not interfere significantly.

False decreased results may occur if heparin is used. Anticoagulants containing citrate, fluorite, or oxalate inhibit Gamma GT activity.

Gamma GT is lower in women than in men at the same age.

Chronic alcoholism increases the Gamma GT activity in serum.

Gamma GT raised levels were observed in patients who used antiepileptic drugs.

Materials required not provided

1. Pipettes to measure reagents and samples
2. Timer

Continuous kinetic method

1. Photometer capable of measuring absorbance at 400 - 420 nm and keeping the cuvette at 37ºC.

Fixed-time kinetic method

1. A constant temperature water bath (37ºC).
2. 5% Acetic Acid solution.
3. Photometer capable of measuring absorbance at 400 - 420 nm.

Preparing the working reagent . Use one bottle of Reagent 1 and Reagent 2 for preparing Working Reagent. Transfer all the contents of one Reagent 2 bottle to one Reagent 1 bottle and mix gently.

The Working Reagent is stable 1 day at 15-25ºC and 21 days at 2-8ºC, when no chemical or microbial contamination occurs.

Procedure

Continuous kinetic method . See Calibration and notes 1, 2 and 3.

Reactions conditions: wavelength: 405 nm; cuvette at 37±0.2ºC, 1cm light path, pass band ≤2nm and stray light ≤0.1.

1. In a test tube labeled "Test" or "Calibrator", add 1.0 mL of the Work Reagent.

2. Add 0.05 mL of the sample or enzyme calibrator, homogenize and transfer immediately to a cuvette at 37±0.2ºC. Wait one minute.

3. Measure the initial absorbance (A_1) at 405 nm (400 - 420 nm), and start simultaneously the timer. Measure the absorbance again after 2 minutes (A_2).

In order to verify the reaction linearity, it is recommended to measure in 1 minute as well, and check if the difference of absorbance in each minute is constant.

Fixed-time kinetic method . See Calibration and notes 1, 2 and 3.
This method requires 5% acetic acid solution.

In one tube, add 0.8 mL of Reagent 1 and 0.2 mL of Reagent 2. Mix and transfer 0.5 mL of the Working Reagent to another tube. Label the tubes as Blank and Test.

	Blank	Test
Working Reagent	0.5 mL	0.5 mL

Incubate at 37ºC during 2 minutes. Do not remove the tubes from the water bath, add:

Sample	-----	0.025 mL

Mix and keep at 37ºC during 10 minutes. Add:

5% Acetic acid	1.0 mL	1.0 mL

Mix and add:

Sample	0.025 mL	-----

Mix and measure the Test absorbance at 405 nm or Blue Filter (400 - 420) against Blank. The color is stable for 60 minutes.

Calibration . Measure the Standard absorbance in triplicate. The difference between the measures must be less than 2%.

	Blank	Standard
Water	0.5 mL	0.5 mL
Standard	-----	0.05 mL
5% Acetic acid	1.0 mL	1.0 mL

Mix and measure the Standard absorbencies at 405 nm (400 - 420 nm) against Blank. The color is stable for 60 minutes.

Calibration

Manual calibrations
Continuous kinetic method
Use Labtest Calibra Series. Gamma GT activity is traceably to reference material ERM-AD452/IFCC and the reference method of IFCC[2].

Calibration frequency
Two or three point calibration after reagent lot change;
Two or three point calibration when the internal quality control indicates.

Fixed-time kinetic method
Use the Standard Ref.: 105.3. The Standard is traceable to reference material ERM-AD452/IFCC and the reference method of IFCC[2].

Perform a new calibration after reagent lot change or when the internal quality control indicates.

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Automatic Systems
Blank of reagents: water or 0.85% NaCl;
Standards: Calibra Series (Labtest calibrator for automated systems), which are traceable to reference material ERM-AD452/IFCC and the reference method of IFCC[2].

Calibration frequency
Two or three point calibration after reagent lot change;
Two or three point calibration when the internal quality control indicates;

Quality control . The limits and control interval must be adapted to the laboratory requirements. Each laboratory should establish corrective measures to be taken if values fall outside the control limits.

It is recommended to use products from Qualitrol line - Labtest for internal quality control in clinical chemistry trials.

Calculations

Continuous Kinetic method

$$\Delta A \text{ (test or calibrator)} = \frac{A_2 - A_1}{2}$$

$$\text{Factor} = \frac{\text{Calibrator activity}}{\Delta A \text{ Calibrator}}$$

Gamma GT activity = ΔA Test x Factor

When the optimum reaction conditions are obtained the 2550 factor can be applied.

Fixed-time kinetic method:

Standard = 125 U/L

$$\text{Gamma GT (U/L)} = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times 125$$

$$\text{Calibration factor} = \frac{125}{\text{Standard absorbance}}$$

Gamma GT (U/L) = Test absorbance X Factor

Linearity

Up to 700 U/L.

If Gamma GT concentration exceeds 700 U/L, the sample must be diluted with 0.85% NaCl. Multiply the result by the appropriate dilution factor.

Dilute the sample so that the obtained value is around 50 and 400 U/L.

Expected values[7,2] . Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

Gamma GT (U/L) - 37ºC

Women	
0 - 6 months	15 - 132
6 - 12 months	1 - 39
1 - 12 years	4 - 22
13 - 18 years	4 - 24
Adults	5 - 39

Gamma GT (U/L) - 37ºC

Men	
0 - 6 months	12 - 122
6 - 12 months	1 - 39
1 - 12 years	3 - 22
13 - 18 years	2 - 42
Adults	7 - 58

Conversion: Conventional Unit (U/L) x 16.7 = SI Unit (nKat/L)

Temperature . The table below allows the conversion of the measured activity at a stated temperature into a value that would be obtained if the measurement was performed in other temperatures.

Work Temperature	Factors for temperature correction		
	25°C	30°C	37°C
25°C	-----	1.37	1.79
30°C	0.73	-----	1.30
37°C	0.56	0.77	-----

Example: the enzymatic activity obtained at 37ºC must be multiplied to the factor 0.77 in order to obtain the activity at 30ºC or to the factor 0.56 in order to obtain the activity at 25ºC.

Performance characteristics[9]

Recovery studies . In two samples with Gamma-Glutamyltransferase activities of 295 and 485 U/L were added different quantities of the enzyme, obtaining the follow results:

Initial Activity (U/L)	295	485
Added Activity (U/L)	101	101
Expected Activity (U/L)	396	586
Obtained Activity (U/L)	391	585
Recovery (%)	98.7	99.8

The mean proportional systematic error at 59 U/L decision level was 0.4 U/L and at 181 U/L decision level was 1.3 U/L.

Method comparison . The proposed method was compared to the reference method, obtaining the follow results:

	Comparison Method	Labtest Method
n	80	80
Range (U/L)	14.7 - 244.0	11.4 - 241.4
Mean (U/L)	119.9	118.8
Regression Analysis	Labtest method = 1.015 x comparison method - 2.97	
Correlation Coefficient	0.999	

The estimated total error is -3.5 % in a decision level of 59 U/L and -0.14 % in a decision level of 181 U/L.

Imprecision Within Run

	N	Mean (U/L)	SD (U/L)	%CV
Sample 1	20	59	0.7	1.13
Sample 2	20	181	1.0	0.57

Imprecision - Run-to-Run

	N	Mean (U/L)	SD (U/L)	%CV
Sample 1	20	59	1.5	2.58
Sample 2	20	181	3.2	1.80

Total error . The estimated total error (random + systematic) in a decision level of 59 U/L is 7.8 % and in a decision level of 181 U/L is 3.1 %.

Analytical sensitivity . Detection limit: 2.48 U/L. The detection limit represents the lowest measurable Gamma-Glutamyltransferase activity that can be distinguished from zero.

Effects of matrix dilution . Samples with values equal 710 and 603 U/L were used for evaluating the system response on dilution with 150 mmol/L NaCl (0.85%). Using dilution factor from 2 to 16, the recovery was 99.0 %.

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The water in the laboratory to prepare reagents and use in the measurements, must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiems/cm and silicates concentration must be ≤0.1mg/L (Type II reagent water). The water for washing must be Type III, having resistivity ≥0.1 megaohms or conductivity ≤10 microsiemens. For the final washing, use Type II reagent water.

3. It is suggested to consult http:/www.fxol.org in order to review physiopathological source and drugs interference in results and methodology.

References

1. Szasz G. Clin Chem 15:124, 1969.
2. IFCC Reference Procedure for the Measurement of Catalytic Concentration of γ-Glutamyltransferase. Clin Chem Lab Med 2002; 40:734-38.
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4. Sociedad Española de Bioquímica Clínica y Patología Molecular, Base de Datos de Variación Biológica. Available at: <http://www.seqc.es/article/articleview/330/1/170> (Access in 04/2006).
5. Basques JC. Especificações da Qualidade Analítica. Labtest Diagnóstica 2005.
6. Westgard JO, Barry PL, Hunt MR, Groth T. Clin Chem. 1981, 27:493-501.
7. Soldin SJ, Brugnara C, Wong EC: Pediatric Reference Intervals, 5.ed. Washington: AACC Press, 2005. p.98-99.
8. Burtis CA, Ashwood ER. Tietz Textbook of Clinical Chemistry, Philadelphia: Saunders Company 1994;980-86.
9. Labtest: data on file.

Presentation

Product	Reference	Contents	
GAMMA GT Liquiform	105-2/30	R 1	2 X 24 mL
		R 2	2 X 6 mL
		CAL	1 X 3 mL
	105-2/50	R 1	2 X 40 mL
		R 2	2 X 10 mL
		CAL	1 X 3 mL
GAMMA GT Liquiform Labmax 560/400	105-4/34	R 1	4 X 27 mL
		R 2	4 X 7 mL
		CAL	1 X 3 mL

The number of tests **in automated instruments depends on the programmed parameters.**

Application procedures using Gamma-Glutamyltransferase **are available for various automated instruments.**

Customer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

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CE

Labtest Diagnóstica S.A.
CNPJ: 16.516.296 / 0001 - 38
Av. Paulo Ferreira da Costa, 600 - Vista Alegre - CEP 33400-000
Lagoa Santa . Minas Gerais Brasil - **www.labtest.com.br**
Customer Service | e-mail: customerservice@labtest.com.br

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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with ivd devices

	**Conteúdo suficiente para < n > testes** Contenido suficiente para < n > tests Contains sufficient for < n > tests		**Risco biológico** Riesgo biológico Biological risk
	**Data limite de utilização (aaaa-mm-dd ou mm/aaaa)** Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)		**Marca CE** Marcado CE CE Mark
	**Material Calibrador** Material Calibrador Calibrator Material		**Tóxico** Tóxico Poison
	**Material Calibrador** Material Calibrador Calibrator Material		**Reagente** Reactivo Reagent
	**Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)		**Fabricado por** Elaborado por Manufactured by
	**Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community		**Número do lote** Denominación de lote Batch code
	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use		**Controle** Control Control
	**Número do catálogo** Número de catálogo Catalog Number		**Controle negativo** Control negativo Negative control
	**Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes		**Controle positivo** Control positivo Positive control
	**Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device		**Controle** Control Control
	**Liofilizado** Liofilizado Lyophilized		**Corrosivo** Corrosivo Corrosive
	**Período após abertura** Período post-abertura Period after-opening		**Uso veterinário** Uso veterinario Veterinary use
	**Instalar até** Instalar hasta Install before		Ref.: 140214

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