γ–GT. Carboxy substrate. Kinetic. Liquid Ref.:GGT-006
Quantitative Determination of gamma-glutamyl transferase (γ–GT)
Only for *in vitro* use in clinical laboratory R1 5 x 40 mL
Store at 2-8ºC R2 1 x 50 mL

γ–GT

CE

PRINCIPLE OF THE METHOD

Gamma-glutamyl transferase (γ-GT) catalyses the transfer of γ-glutamyl group from γ-glutamyl-p-nitroanilide to acceptor glycylglycine, according to the following reaction:

γ--L-Glutamyl-3-carboxy-4-nitroanilide + Glycylglycine $\xrightarrow{\gamma\text{–GT}}$ γ-L-Glutamyl-glycylglycine + 2-Nitro-5-aminobenzoic acid

The rate of 2-nitro-5-aminobenzoic acid formation, measured photometrically, is proportional to the catalytic concentration of γ-GT present in the sample[1,2].

CLINICAL SIGNIFICANCE

Gamma-glutamyl transferase (γ-GT) is a cellular enzyme with wide tissue distribution in the body, primarily in the kidney, pancreas, liver and prostate.
Measurements of gamma-glutamyl transferase (γ-GT) activity are used in the diagnosis and treatment of hepatobiliary diseases such biliary obstruction, cirrhosis or liver tumours[1,2,5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	TRIS pH 8.6 Glycylglycine	100 mmol/L 100 mmol/L
R 2 Substrate	L-γ-glutamyl-3-carboxy-4-nitroanilide	3 mmol/L

PREPARATION

Working reagent (WR)
Mix: 4 vol. (R1) Buffer + 1 vol. (R2) Substrate

Stability: 21 days at 2-8ºC or 5 days at room temperature (15-25ºC).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 405 nm ≥ 1.20.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 25ºC, 30ºC o 37ºC (± 0.1ºC)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum[1]. γ–GT is stable for at least 3 days at 2-8ºC, 8 hours at 15-25ºC and 1 month at – 20ºC.

PROCEDURE

1. Assay conditions:
   Wavelength: . . . . . 405 nm
   Cuvette: . . . . . 1 cm light path
   Constant temperature . . . . . .25ºC /30ºC / 37ºC
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette(note 1):

WR (mL)	1.0
Sample (µL)	100

4. Mix, wait for 1 minute.
5. Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1 minute intervals thereafter for 3 minutes.
6. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

CALCULATIONS

ΔA/min x 1190 = U/L of γ-GT

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Temperature conversion factors
To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25ºC	30ºC	37ºC
25ºC	1.00	1.37	1.79
30ºC	0.73	1.00	1.30
37ºC	0.56	0.77	1.00

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

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

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 2 U/L to linearity limit of 250 U/L.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (U/L)	38.0	188	37.5	190
SD	0.79	2.57	0.96	2.61
CV (%)	2.09	1.36	2.56	1.37

Sensitivity: 1 U/L = 0.0074 ΔA/min.
Accuracy: Results obtained using BSM reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 100 samples were the following:
Correlation coefficient (r): 0.9960.
Regression equation: y= 0.9897x - 0.0879.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Plasma should not be used, anticoagulants inhibit the enzyme. Gross haemolysis interferes in the assay[1].
A list of drugs and other interfering substances with γ-GT determination has been reported by Young et. al[3,4].

NOTES

BSM has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

BIBLIOGRAPHY

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