γ - GT

Carboxy-glupa method

R1: 2 x 40 ml + R2: 2 x 10 ml CL34-100
R1: 2 x 80 ml + R2: 2 x 20 ml CL34-200

INTENDED USE

Kit for quantitative determination of γ-Glutamyl-Transferase, γ-GT (EC 2.3.2.2.) in serum and plasma according to Szaz modified method.

CLINICAL MEANING

γ-Glutamyl-Transferase (GGT) is an enzyme which carries amino acids through cellular membranes. The highest concentrations of this enzyme are in hepatic tissue and in the biliary tract. The measurement is used to identify a hepatic cellular dysfunction (hepatitis, cirrhosis, neoplasia) and also to identify minor cholestasis.

PRINCIPLE

In presence of glycyl-glycine, γ-GT splits L-γ-glutamyl-3-carboxy-4-nitroanilide (carboxy-glupa) in L-γ-glutamyl-glycyl-glycine and 5-amino-2-nitro-benzoate. The absorbance change in time unit measured at 405 nm is proportional to the enzyme activity in the sample.

SAMPLE

Serum, EDTA or heparinized plasma. Do not use hemolyzed samples.
Stability: 1 week at 2-8 °C.

REAGENTS

Only for in Vitro diagnostics. Liquid reagents ready to use.

Package content	CL34-100	CL34-200
REAGENT 1 Tris buffer (pH 8,3) 100 mmol/L, glycil-glycine 100 mmo/L, sodium azide 15 mmol/L	2 x 40 ml	2 x 80 ml
REAGENT 2 Tris buffer (pH 6,3) 10 mmol/L, L-γ-glutamyl-3-carboxy-4-nitroanilide 20 mmol/L, sodium azide 15 mmol/L	2 x 10 ml	2 x 20 ml

Stability: Store at 2-8°C and protect from light to keep the reagents stable up to the expiration date on the label. Once opened the reagents are stable for 2 months at 2-8°C if contamination is avoided. Keep bottles closed when not in use. Do not use turbid reagents.

NECESSARY ITEMS – NOT PROVIDED

Usual laboratory equipment: UV/VIS Spectrophotometer with temperature control; automatic micropipettes; Optical glass cuvettes or, alternatively, disposable ones in optical polystyrene; Saline solution.

REAGENT PREPARATION

(only for monoreagent application)

To prepare the working reagent, mix 4 volumes of Reagent 1 with 1 volume of Reagent 2.
Stability: 5 days at 20-25°C or 4 weeks at 2-8°C if stored in a closed bottle protected from light.

MANUAL ASSAY PROCEDURE

Method:	increasing kinetic
Wavelength:	405 nm
Optical path:	1 cm
Temperature:	25, 30, 37°C
Reading time:	3 minutes
Reading:	against air or distilled water
Sample/reagent ratio (bireagent):	1/10/2,5
Sample/reagent Ratio (monoreagent):	1/10

BIREAGENT PROCEDURE

Bring the working reagent to the chosen temperature for the analysis.
Pipette in cuvette:

Sample	100 µl
Reagent 1	1,0 ml

Mix and incubate 1 minute at 37°C. Add:

Reagent 2	250 µl

Mix and pour into cuvettes. Incubate at the chosen temperature for 1 minute. Read initial absorbance, repeat the reading at constant intervals of one minute for 3 minutes. Calculate the average value of absorbance variations per minute (ΔA/min).

Reaction volumes can be proportionally varied without change in calculation.

MONOREAGENT PROCEDURE

Bring the reagents to the chosen temperature for the analysis.
Pipette in cuvette:

Sample	100 µl
Working reagent	1,0 ml

Mix and pour into cuvettes. Incubate at the chosen temperature for 1 minute. Read initial absorbance, repeat the reading at constant intervals of one minute for 3 minutes. Calculate the average value of absorbance variations per minute (ΔA/min). Reaction volumes can be proportionally varied without change in calculation.

CALCULATION

Calculate the enzymatic activity in the sample multiplying ΔA/min by the proper factor from the following table.

Monoreagent procedure	Bireagent procedure
1158	1421

REFERENCE VALUES

	25°C	30°C	37°C
Men	5 ÷ 25	7 ÷ 35	10 ÷ 50
Women	5 ÷ 18	6 ÷ 25	8 ÷ 35

Each laboratory should define its own reference values for this method.

QUALITY CONTROL – CALIBRATION

All Clinical Chemistry laboratories should implement a quality control program. Control serums of human origin are available for this purpose on request:
PRE-NORM serums with normal values
PRE-PATH serums with pathological values
If the method requires it, a multiparameter calibrator of human origin is available.

PERFORMANCE CHARACTERISTICS

Sensitivity: the sensitivity of the method is 3 U/L.

Linearity: up to 230 U/L (at 37°C).
For higher values, dilute the samples 1:10 with saline solution and multiply the result by 10.

Precision:

Within run (n=10)	Average [U/L]	SD	CV %
Sample 1	35,1	0,5	1,5
Sample 2	170,1	0,5	0,3

Between run (n=20)	Average [U/L]	SD	CV %
Sample 1	35,4	0,7	2
Sample 2	167,1	2,8	1,7

Interferences: up to 30 mg/dl of bilirubin does not interfere. Up to 50 mg/dl of ascorbic acid does not interfere. Proportionally to its presence in the sample, hemolysis may give falsely reduced values.

Correlation against a reference method: the correlation of FAR method (Y) against a reference method (X) gives this equation:

Y = 1,0399X + 2,1657 r = 0,9994

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

Contact with the skin and ingestion should be avoided. Use the normal precautions expected with correct behavior in laboratory.

REFERENCES

1. Recommendation on I.F.C.C. methods for measurement of catalytic concentrations of enzymes, Clin Chem, 23:5 (1977)
2. Wroblewsky F., Ladue J.S., Proc. Soc. Exper. Biol and Med, 91:569 (1965)
3. Kaplan LA, Pesce AJ: "Clinical Chemistry", Mosby Ed. 1989

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalog number
	temperature limits
	use by
	caution
	consult accompanying documents

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