γ-GLUTAMYL TRANSFERASE KIT LS

(Carboxy Substrate Method)

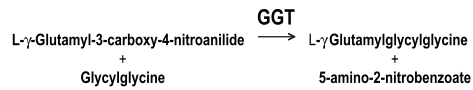
For the determination of γ-Glutamyl Transferase activity in serum.
(For Invitro Diagnostic Use Only)

Summary

γ-Glutamyl Transferase (GGT) is an enzyme found mainly in serum from hepatic origin, though the highest levels are in kidneys. Elevated levels are found in hepatobiliary and pancreatic diseases. Chronic alcoholism, myocardial infarction with secondary liver damage and diabetics.

Principle

GGT catalyzes the transfer of amino group between L-γ-Glutamyl-3-carboxy-4-nitroanilide and Glycylglycine to form L-γ-Glutamylglycylglycine and 5-amino-2-nitrobenzoate. The rate of formation of 5-amino-2- nitrobenzoate is measured as an increase in absorbance which is proportional to the GGT activity in the sample.



Normal Reference Values

Serum (Males) : 10 - 50 U/L at 37°C
(Females) : 7 - 35 U/L at 37°C

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	25 ml	75 ml
L1 : Buffer Reagent	20 ml	60 ml
L2 : Starter Reagent	5 ml	15 ml

Storage / Stability

Contents are stable at 2-8° C till the expiry mentioned on the labels.

Reagent Preparation

Reagents are ready to use.

Working reagent : For sample start assays a single reagent is required. Pour the contents of 1 bottle of L2 (Starter Reagent) into 1 bottle of L1 (Buffer Reagent). This working reagent is stable for at least 2 weeks when stored at 2-8° C.

Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of L1 (Buffer Reagent) and 1 part of L2 (Starter Reagent). Alternatively 0.8 ml of L1 and 0.2 ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

Sample Material

Serum. Free from hemolysis. GGT is reported to be stable in serum for 3 days at 2-8° C.

Procedure

Wavelength / filter : 405 nm
Temperature : 37° C / 30° C / 25° C
Light path : 1 cm

Substrate Start Assay :

Pipette into a clean dry test tube labelled as Test (T) :

Addition Sequence	(T) 37°C
Buffer Reagent	0.8 ml
Sample	0.1 ml
Incubate at the assay temperature for 1 minute and add	
Starter Reagent	0.2 ml

Mix well and read the initial absorbance A_0 after 30 seconds & repeat the absorbance reading after every 1 & 2 minutes. Calculate the mean absorbance change per minute (ΔA / min.)

Sample Start Assay :

Pipette into a clean dry test tube labelled as Test (T) :

Addition Sequence	(T) 37°C
Working Reagent	1.0 ml
Incubate at the assay temperature for 1 minute and add	
Sample	0.1 ml

Mix well and read the initial absorbance A_0 after 30 seconds & repeat the absorbance reading after every 1 & 2 minutes. Calculate the mean absorbance change per minute (ΔA / min.)

Calculations

GGT Activity in U/L = ΔA/ min. x 1158

TEMPERATURE CONVERSION FACTORS

Assay Temperature	Desired Reporting Temperature 25°C	30°C	37°C
25°C	1.00	1.37	1.79
30°C	0.73	1.00	1.30
37°C	0.56	0.77	1.00

Linearity

The procedure is linear upto 700 U/L at 37° C. If the absorbance change (ΔA / min) exceeds 0.250, dilute the sample 1 + 9 with normal saline (NaCl 0.9%) and repeat the assay (Results x 10).

Note

Samples having a very high activity show a very high initial absorbance. If this is suspected then dilute the sample and repeat the assay.

References

IFCC methods for the measurement of catalytic concentrations of enzymes, J. Clin. Chem. Clin Biochem. (1986) 24 : 497

System Parameters

Reaction	:	Kinetic
Wavelength	:	405 nm
Zero Setting	:	Distilled water
Incub. Temp.	:	37^0C
Incub. Time	:	---
Delay Time	:	30 sec.
Read Time	:	120 sec.
No. of read.	:	4
Interval	:	30 sec.
Sample Vol.	:	0.10 ml
Reagent Vol.	:	1.00 ml
Standard	:	---
Factor	:	1158
React. Slope	:	Increasing
Linearity	:	700 U/L
Units	:	U/L

GGT(LS):00(P)

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