CATACHEM

γ –GLUTAMYL-TRANSFERASE (γ -GT)
MANUAL OR AUTOMATED APPLICATIONS
V314-0A (Carboxy GPNA)

Intended Use
For **IN VITRO** quantitative determination of (GGT), ***γ-*** Glutamyltranferase (transpeptidase) (***γ*** GT) in serum or plasma.

Clinical Significance
Measurements of GGT activity are primarily used for diagnosing intra or post hepatic biliary obstructions, metastatic neoplasm, drug intoxication, chronic pancreatitis, as well as for monitoring the causes and treatments.

Method History
In 1960, Goldberg et al (1) Szewczuk and Orlowski (2), reported GGT enzyme activity in serum. Szasz (3) reported the use of gamma glutamyl-p-nitro-anilide (GPNA) as substrate. This substrate, although effective, was difficult to solubilize in an aqueous reagent and was replaced by a carboxylated form of GPNA, Carboxy GPNA (L-***γ***-glutamyl-3-carboxy-4-nitroanilide) which greatly improved solubility and performance of the reagent on automated instruments.

Method Principle
γ-glutamyltranspeptidase transfers the gamma glutamyl group from the substrate Carboxy GPNA to glycyl glycine to release p-nitroaniline which has maximum absorbance at 405nm. The rate of increase in absorbance is directly proportional to the GGT enzyme activity in the serum sample. The reaction scheme illustrates the reaction that occurs in this method.

$$\text{Carboxy GPNA} + \text{glycylglycine} \xrightarrow{\gamma\text{-GT}} \text{Carboxy GPNA-glycylglycine} + \text{p-nitroaniline}$$

Reagent Content
The concentrations of the active ingredients in the reagents will be approximately as follows:

GGT Diluent

Each vial contains:	
Buffer	
Glycylglycine	95.4 mmol
Nonreactive ingredients	

GGT Substrate

Each vial contains:	
Carboxy GPNA	250 mmol
Nonreactive ingredients and stabilizer	

Precautions
Avoid contact of reagents with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Reagent Preparation
Catachem GGT reagents require no preparation and are used as is.

Reagent Storage And Stability
Store the separate GGT reagent components at 2-8˚C. When stored as directed, these reagent components are stable until the expiration date stated on the label. Although usually used as a two reagent system if a single GGT Working Reagent is prepared (9 parts R1 to 1 part R2) this working reagent is stable for at least 15 days if stored at 2-8 ˚C and capped when not in use.

Specimen Collection And Preparation
Test sera should be fresh, clear and unhemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay. In serum the GGT enzyme is stable for approximately one week at 2-8˚C.

Precautions
Avoid contact of the specimen with skin and eyes. Should contact occur, wash affected area with plenty of water. **DO NOT PIPETTE REAGENTS OR SPECIMENS BY MOUTH.**

Quality Control
To monitor the performance of the Reagents and the procedures used, we recommend the regular use of a normal and abnormal control serum.

Interfering Substances
Several substances have been reported to interfere with the GGT method. Elevated values in patients taking antiepileptic drugs such as phenytoin and barbiturates have been reported. Hemoglobin at concentrations greater than 150 mg/dL produces a clinically significant interference with the GGT method. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al.(4)

Expected Values
The range of expected values determined for this method in humans is 0-65 U/L. Values are species dependent. It is recommended that each laboratory establish the normal range for the species under test and for the area in which the laboratory is located.

Procedure
Important: Read the entire procedural instructions before proceeding with the assay.

Materials Required (But Not Provided)

Spectrophotometer	
Matched cuvettes	1 cm light path
Timer	to time incubation time
Pipette	1.0 ml for reagent
Pipette	0.1 ml for sample
Graduated cylinder	25 ml or 50 ml for reagent

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Materials Provided
GGT Reagent

Analytical Parameters

Wavelength	405 nm
Temperature	37˚C
Path length	1 cm
Reaction Mode	rate
Reaction Time	5 minutes
Reagent Volume	1.0 ml
Sample Volume	0.1 ml
Total Volume	1.1 ml
Sample-to-reagent ratio	1:11

Assay Procedure (Assumes a single working reagent when used manually)

1. Set spectrophotometer wavelength at 405nm and zero the instrument with the cuvette containing water.
2. Pipette 1.0 ml of Working Reagent into the number of cuvettes required marked appropriately- "Sample", "Control" etc.
3. Incubate cuvettes for 2 minutes at 37˚C.
4. Pipette 0.1 ml of Control and each Sample into their respective cuvettes. Mix all cuvettes well.
5. Replace the cuvettes in spectrophotometer and continuously monitor the change in absorbance for at least 5 minutes.
6. Measure the "Control" and "Sample" delta absorbencies per minute.
7. Calculate the GGT concentration (U/L) in the sample(s), as shown in results and calculations.

Results And Calculations

$$\text{GGT(U/L)} = \frac{\text{Delta A/min} \times 1.1\text{ml} \times 1000}{9.5 \times \text{L(cm)} \times \text{Sample volume}}$$

Where:

Delta A/min	= change in absorbance per minute
1.1 ml	= total volume in cuvette
0.1 ml	= volume of sample being assayed
9.5	= Extinction coefficient
L	= Path Length in cm
1000	= converts U/ml to U/L

Example: Delta A/min = 0.01

$$\text{GGT (U/L)} = \frac{0.01}{} \times \frac{1}{9.5} \times \frac{1.1\text{ ml}}{0.1\text{ ml}} \times 1000$$

$$= 11.6 \text{ U/L}$$

Method Performance Characteristics
Sensitivity: 0.00085 - 0.0015 absorbance units/U/L.
Linear Range: 0-500 U/L (depends on sample/reagent ratio)
Precision: Within-run and day-to-day precision is summarized below using 10 replicates of each level:

Precision Study

γ-GT	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
U/L	U/L	%	U/L	%
20.1	0.97	4.8	1.2	5.9
70.2	2.85	4.1	3.2	4.6
484	1.90	0.40	4.40	0.90

Correlation
A comparison of this method using an automated analyzer and a referenced procedure resulted in the following regression statistics.

N	=	213
Y	=	1.01 x + 4.1
r	=	0.998
Sy.x	=	3.6

References

1. Goldberg JA, Friedman OM, Riveda EP, Smith EE, Chatterzi R, Stein EH, Rutenberg A. The colorimetric determination of gamma glutamyltranspeptidase with synthetic substrate. Arch Biochem Biophys 91:61-70 (1960).
2. Szewczuk A, Orlowski M. The use of a (N-gamma-DL-glutamyl) aminoitrile for the colorimetric determination of a specific peptidase in blood serum. Clin Chem Acta 5:680-688 (1960).
3. Szasz G. A kinetic photometric method for serum gamma glutamyl transpeptidase.] Clin Chem 15:112-136 (1969).
4. Young DC, Pestaner LC, Gibberman V. Clin Chem 21, 203D (1975).

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