



Stanbio γ -GT (LiquiColor®) Procedure No. 2960

For the Kinetic Quantitative Determination of γ -Glutamyl Transferase in Serum for Manual and/or Automated Procedures

Summary and Principle

Gamma-glutamyl transferase (γ -GT) is one of a large group of enzymes known as peptidases. Although renal tissue has the highest level of γ -GT, the major source of the enzyme present in serum is of hepatic origin. Elevated levels of γ -GT are found in association with hepatobiliary and pancreatic disorders; alcoholics and heavy drinkers, in myocardial disorders and in diabetics.¹

Unlike alkaline phosphatase activity, the serum γ -GT activity remains normal in diseases affecting bone and during normal bone growth. Therefore, a rise in serum γ -GT activity may be considered as a sensitive and more specific indicator of liver disease than alkaline phosphatase activity. The Stanbio γ -GT procedure has been optimized according to Szasz.



L - γ - glutamylglycylglycine + 5 - amino - 2 - nitrobenzoate

γ -GT catalyzes the transfer of a γ -Glutamyl group from L- γ -glutamyl-3-carboxy-4-nitroanilide. The rate of liberation of 5-amino-2 nitrobenzoate is directly related to the γ -GT activity in the sample and is quantitated by measuring the increase in absorbance at 405 nm.

Reagent

γ -GT Buffer (R1), Ref. No. 2961

Composition:

Tris, pH 8.25	100 mmol/L
Glycylglycine	100 mmol/L

γ -GT Substrate (R2), Ref. No. 2962

Composition:

L- γ -Glutamyl-3-Carboxy-4-Nitroanilide, 4.0 mmol/L

Precautions: The reagents are for "In Vitro Diagnostic Use". Normal precautions exercised in handling laboratory reagents should be followed. The reagents contain sodium azide which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

Reagent Preparation: Buffer and Substrate liquid reagents are supplied ready-to-use. Prepare Working Reagent in the ratio of 5 parts Buffer (R1) to 1 part Substrate (R2), (i.e., 25 mL Buffer and 5 mL Substrate).

Reagent Storage and Stability: Reagents are stable until the expiration date on their respective labels, when properly stored at 2-8°C and protected from light. R1 should appear clear/colorless while R2 should appear clear/yellow. Discard if either appears cloudy or contains particulate matter. The Working Reagent is stable for 4 weeks at 2-8°C or 5 days at room temperature (15-25°C). The Working Reagent should be discarded if the initial absorbance, read against distilled water at 405 nm, is above 0.800.

Material Required But Not Provided

Spectrophotometer capable of absorbance reading at 405 nm and 1 cm lightpath
Constant temperature block/bath, 37°C, or temperature controlled cuvette, Accurate pipetting devices, Test tubes and Interval timer

Specimen Collection and Storage

Serum or EDTA plasma, free of hemolysis, should be used. Complexing anticoagulants such as citrate, oxalate, fluoride and must be avoided since they inhibit γ -GT activity.² The loss of γ -GT activity is minimized by storing the samples refrigerated for up to 7 days or frozen up to 2 months.³ Bilirubin levels up to 40 mg/dL and triglyceride levels up to 2000 mg/dL show no interference in this test.⁸

Interfering Substances: γ -GT is an inducible enzyme. Consequently patients who are receiving antiepileptic drugs or aminopyrine show elevated γ -GT activity.^{4,5} Chronic use of ethanol also increases serum γ -GT activity. Certain drugs and other substances are also known to affect γ -GT values.⁶

Manual Procedure

1. Prepare γ -GT Working Reagent according to instructions.
2. Zero spectrophotometer at 405 nm with distilled water.
3. For each sample and control, add 1.0 mL Working Reagent to cuvette or test tube and warm to 37°C for 3 minutes.
4. Add 100 μ L (0.10 mL) serum to its respective tube and mix gently.
5. Read and record absorbance at 1 minute. Continue incubating at 37°C and record absorbance again at 2 and 3 minutes. Rate should be constant.
6. Determine the average absorbance per minute ($\Delta A/\text{min}$), multiply by factor 1158 for results in U/L.

NOTE: If cuvette is not temperature controlled, incubate samples at 37°C between readings.

Automated Procedure

Special adaptations for automated analyzers are available by contacting Stanbio's Customer Service Department.

Quality Control: Stanbio Ser-T-Fy® I, Normal Control Serum, Cat. No. G427-86 and Stanbio Ser-T-Fy® II, Abnormal Control Serum, Cat. No. G428-86 are recommended for verifying accuracy and precision. Other commercially available controls with γ -GT values assayed by this method are also suitable. γ -GT activity determined in these materials, by this procedure should fall within the ranges stated for the controls. Two levels of controls should be analyzed each day of testing.

Calibration: γ -GT activity is based on the "micromolar extinction coefficient" of 5-amino-2-nitrobenzoate at 405 nm (see "Results" section). The instrument manufacturer's calibration guidelines should be followed to calibrate your analyzer.

Results

Values are derived based on the "absorptivity micromolar extinction coefficient" of 5-amino-2-nitrobenzoate at 405 nm (0.0095). Units per liter (U/L) γ -GT activity is that amount of enzyme which products one mmol/L of 5-amino-2-nitrobenzoate per minute.

$$U/L = \frac{\Delta A / \text{Min}}{\text{Absorptivity}} \times \frac{\text{Total Volume}}{\text{Sample Volume}}$$

$$U/L = \frac{\Delta A / \text{Min}}{0.0095} \times \frac{1.10}{0.10}$$

$$U/L = \Delta A/\text{Min} \times 1158$$

Limitations

If the $\Delta A/\text{min}$. is greater than .515, dilute 1 part sample with 5 parts isotonic saline and re-assay. Multiply results by 6.

Expected Values⁷

Normal Range: Males: 0 - 50 U/L (37°C)
Females: 0 - 30 U/L (37°C)

This range should serve only as a guideline. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

Performance Characteristics

Comparison: A group of 63 sera ranging in γ -GT activity from 15 - 714 U/L was assayed by the described γ -GT method and by a similar commercially available γ -GT reagent. Comparison of the results yielded a correlation coefficient of 1.000 and the regression equation was $y = 0.94x + 1.9$. (Comparison studies were performed according to NCCLS Tentative Guideline, EP9-T.)

Precision: Within-run precision was established by 20 assays on three different levels of commercial serum controls. Total Precision values were obtained by assaying the 3 commercial controls for 5 consecutive days.

	Within-Run		
	Serum 1	Serum 2	Serum 3
Mean γ -GT (U/L)	40	74	206
Std. Deviation (U/L)	1.0	0.9	1.3
C.V. (%)	2.5	1.2	0.6

	Total Precision		
	Serum 1	Serum 2	Serum 3
Mean γ -GT (U/L)	42	72	204
Std. Deviation (U/L)	0.6	0.6	0.7
C.V. (%)	1.5	0.9	0.4

Precision studies were performed according to NCCLS Tentative Guideline, EP5-T.

Linearity: Linear to 300 U/L at 37°C.⁸ Performed according to NCCLS Guideline EP6-P.

Sensitivity: Based on an instrument resolution of A = 0.001, the method presented shows a sensitivity of 1.0 U/L.

References

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8. Stanbio Laboratory Data

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