

Gamma GT

1.0 INTENDED USE

This reagent is intended for the quantitative determination of Gamma GT in serum.

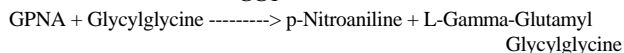
2.0 BACKGROUND

2.1 METHOD AND HISTORY

The enzyme Gamma-Glutamyltransferase (GGT) was first demonstrated in human serum by Goldbarg (10.1) and Szewczuk and Orłowski (10.2) in 1960. These investigators described a GGT assay using a synthetic substrate. Szasz (10.3) modified the procedure, and optimized the concentrations of the substrate and peptide acceptor. The Biotron method is based on the optimized conditions published by Szasz.

2.2 TEST PRINCIPLE

GGT catalyzes the transfer of a Gamma-Glutamyl group from Gamma-Glutamyl-p-Nitroanilide (GPNA) to Glycylglycine to form Gamma-Glutamyl-Glycylglycine and p-Nitroaniline.



The rate of increase in absorbance at 405nm due to the formation of p-nitroaniline is directly proportional to the GGT activity.

2.3 CLINICAL SIGNIFICANCE

The enzyme Gamma-Glutamyltransferase (GGT) occurs in highest concentrations in the kidney, liver and pancreas, but it is also found in the prostate, salivary glands, brain and heart. Serum GGT is generally elevated as a result of liver disease. Serum GGT is elevated earlier than other liver enzymes in diseases such as acute cholecystitis, acute pancreatitis, acute and subacute liver necrosis. Elevated GGT levels also help to differentiate between liver and bone diseases when measured in conjunction with alkaline phosphatase. The measurement of GGT has also been advocated as a screening test for alcohol abuse.

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen. Lithium heparinized plasma may be used as a specimen. Other anticoagulants should be avoided.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-200 µl. Call Biotron's technical service department at 1-800 595 8766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

Serum samples may be stored at 2°-8°C for up to 7 days, and for two months at -20°C (10.4). Frozen samples should be thawed at room temperature and mixed completely before analysis. Thawed samples should not be refrozen.

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

4.0 MATERIALS

(10 X 10 ml)

(6 X 50 ml)

(6 X 100 ml)

Reagents necessary for the determination of Gamma GT are included in the kit.

4.1 REAGENT

GGT GPNA Reagent contains, after reconstitution with deionized water:

L-Gamma-Glutamyl-p-Nitroanilide ≥ 2.9 mM

glycylglycine ≥ 50 mM

2-amino-2-methyl-1,3-propanediol ≥ 100 mM

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

4.3 REAGENT PREPARATION

4.3.1

The working reagent is prepared by reconstituting each vial of GGT GPNA reagent with 10 ml of deionized water. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

4.3.2

The working reagent is prepared by reconstituting each vial of GGT GPNA reagent with 50 ml of deionized water. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

4.3.3

The working reagent is prepared by reconstituting each vial of GGT GPNA reagent with 100 ml of deionized water. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

4.4 REAGENT STORAGE AND STABILITY

When stored at 2°-8°C unopened reagents are stable until the expiration date printed on the label.

The working reagent is stable for 5 days at 18°-26°C or until the absorbance is greater than 0.800 read against deionized water at 405 nm. A precipitation may appear at temperatures below 18° C. Warm the reagent to 50° to 60° C to dissolve the precipitated material.

The working reagent should be clear. Cloudiness indicates contamination and the reagent should be discarded. The absorbance should be less than 0.800 read against deionized water at 405 nm.

4.5 ADDITIONAL MATERIALS REQUIRED

4.5.1 Spectrophotometer or colorimeter capable of reading absorbance at 405 nm.

4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 405 nm.

4.5.3 Test tubes capable of holding 3 ml.

4.5.4 Pipettes capable of delivering 2 ml and 200 µl.

4.5.5 Timer for a 1 minute intervals.

4.5.6 Constant temperature source which can be adjusted to 30° or 37° C.

4.5.7 Normal and abnormal controls for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

Wavelength	405 nm
Temperature	30° or 37° C
Pathlength	1.0 cm
Mode	kinetic
Reaction time	2-4 min
Sample volume	200 µl
Reagent volume	2 ml
Total volume	2.2 ml
Sample to reagent ratio	1/10

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 405 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

No reagent calibration is necessary as this method is standardized by means of the molar absorptivity of p-Nitroaniline taken as 9.90 at 405nm under the test conditions described.

5.4 PROCEDURE

5.4.1 Prepare the required volume of working reagent (see 4.3 Reagent Preparation Section.)

5.4.2 Into separate test tubes pipette 200 µl of serum to be assayed.

5.4.3 Add 2.0 ml of reagent, mix and incubate for 2 minutes at 30° or 37° C. The lag time will be decreased if the reagent is prewarmed to the incubation temperature.

5.4.4 Record the change in absorbance at 405 nm at one minute intervals until the absorbance change is constant.

5.5 CALCULATION AND RESULTS

G-GT (U/L) = $\Delta A/\text{min} \times 1111$

$\Delta A/\text{min} \times \text{assay volume (ml)} \times 1000$

$= \frac{\Delta A/\text{min} \times \text{assay volume (ml)} \times 1000}{\text{assay volume (ml)}}$

$9.9 \times \text{light path (cm)} \times \text{sample volume (ml)}$

1111 = factor derived from the constants in the equation

$\Delta A/\text{min}$ = change in absorbance per minute

assay volume = 2.2 ml

1000 = converts U/ml to U/L

9.9 = molar absorptivity of p-nitroaniline at 405 nm

light path = 1 cm

sample volume = 0.2 ml

Example:

G-GT U/L = $0.025 \times 1111 = 28 \text{ U/L}$, with $\Delta A/\text{min} = 0.025$.

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES (10.3)

The range of expected values for the GAMMA GT method:

Male: 7-40 U/L at 30° C, 9-52 U/L at 37° C

Female: 4-25 U/L at 30° C, 5-32 U/L at 37° C

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

6.2 MEDICAL ALERT VALUES (10.6)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

6.3 LIMITATIONS OF PROCEDURE

Oxalates, fluoride and citrate have been found to inhibit the reaction. Some antiepileptic drugs (phenytoin, barbiturates) may falsely elevate GGT values.(10.3)

Young (10.5) gives a list of drugs and other substances that interfere with the determination of GGT activity.

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

No routine reagent calibration is necessary as this method is standardized by means of the molar absorptivity of p-Nitroaniline taken as 9.90 at 405nm under the test conditions described.

The results obtained when measuring the activity of a kinetic reaction are based on the change in absorbance per minute. In order to accurately monitor and report this reaction rate, the operating parameters of the spectrophotometer (wavelength, temperature of the reaction and timing of the test) must be known and controlled.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run

In this study, 15 replicates of 2 control sera were run.

<u>Mean (U/L)</u>	<u>SD (U/L)</u>	<u>CV (%)</u>
39	± 0.44	1.13
148	± 1.20	0.81

Between-Run

In this study, 5 runs were made, each run consisting of 10 replicates of 2 control sera.

<u>Mean (U/L)</u>	<u>SD (U/L)</u>	<u>CV (%)</u>
40	± 0.43	1.07
148	± 1.06	0.72

9.2 CORRELATION

A correlation study was done comparing this method (y) with a similar comparative method (x) on a Technicon instrument operating at 37° C. 45 samples were run with a range between 12 U/L and 941 U/L.

<u>Number of Samples</u>	<u>Regression Equation</u>	<u>Correlation Coefficient</u>
45	$y = 1.094x - 8.65$	0.999

9.3 LINEARITY

This procedure is linear through 750 U/L. Procedures on automated instruments which use greater than one to ten (sample to reagent) dilution factor will have an extended linearity.

A sample with Gamma GT beyond the linearity limit should be diluted 1 to 1 with 0.9% saline. Reassay the specimen and multiply the results by 2.

9.4 SENSITIVITY

A change in absorbance of 0.001 $\Delta A/\text{min}$ at 405nm at 37° C corresponds to 1.1 U/L.

10.0 REFERENCES

- 10.1 Goldbarg, J.A., Friedman, O.M., Reveda, E.P., and Smith, E.E., Arch Biochem Biophys, 91,61(1960)
- 10.2 Szewczuk, A., Orlovski, M., Clin Chem Acta, 5,680(1960)
- 10.3 Szasz, G., Clin Chem, 15,112(1969)
- 10.4 Kaplan, L., Pesce, A., Clinical Chemistry Theory, C.V. Mosby, Princeton, NJ (1984)
- 10.5 Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., Washington DC, AACC Press (1990).
- 10.6 G.J. Kost, "Critical Limits for Urgent Clinician Notification at U.S. Medical Centers"; JAMA, Feb. 2, 1990; Vol 263, No.5, p.704

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