



## $\gamma$ -GLUTAMYLTRANSFERASE-SL ASSAY

**CATALOGUE NUMBER:** 324-10  
324-30  
324-50A  
324-50B

**SIZE:** R1: 1 x 100 mL, R2: 1 x 25 mL  
R1: 3 x 100 mL, R2: 1 x 75 mL  
R1: 1 x 1000 mL  
R2: 1 x 300 mL

### INTENDED USE

For the quantitative determination of  $\gamma$ -GT in serum. For IN VITRO diagnostic use.

### PRECAUTIONS

Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.

### REAGENTS

$\gamma$ -GT-SL Buffer Reagent (R1): A solution containing buffer (pH 8.3 at 25°C), 100 mmol/L glycylglycine and a preservative.

$\gamma$ -GT-SL Substrate Reagent (R2): A solution containing 4 mmol/L L- $\gamma$ -glutamyl-3-carboxy-4-nitroanilide.

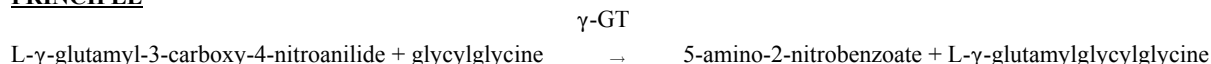
### HISTORY

$\gamma$ -Glutamyltransferase ( $\gamma$ -GT) was initially purified and characterized by Szewczuk and Baromouski (1).  $\gamma$ -GT catalyzes the transfer of the  $\gamma$ -glutamyl group from a  $\gamma$ -glutamyl peptide to an amino acid of another peptide.

Measurements of its activity are used in diagnosis of liver diseases such as alcoholic cirrhosis and primary and secondary liver tumors. (2)

Measurement of  $\gamma$ -GT using L- $\gamma$ -glutamyl-P-nitroanilide as a substrate was introduced by Orłowski and Meizler (3) and adapted for determinations in serum by Szasz (4). The method was modified by Persijn and Vanderslik (5) to use a more soluble L- $\gamma$ -glutamyl-3-carboxy-4-nitroanilide substrate. Reagents in this assay use the carboxylated substrate for detection of  $\gamma$ -GT following a modification of the International Federation of Clinical Chemistry rapid kinetic procedure for serum  $\gamma$ -GT (6).

### PRINCIPLE



Gamma glutamyl transferase catalyzes the transfer of the glutamyl group from L- $\gamma$ -glutamyl-3-carboxy-4-nitroanilide to glycylglycine, forming L- $\gamma$ -glutamylglycylglycine and 5-amino-2-nitrobenzoate. The 5-amino-2-nitrobenzoate absorbs strongly at 405 nm. Formation of this product is proportional to  $\gamma$ -GT activity and is measured kinetically at 405 nm.

### REAGENT PREPARATION

The reagents are provided in a ready to use format. A single working reagent may be prepared by combining 4 parts  $\gamma$ -GT-SL Buffer Reagent (R1) with 1 part  $\gamma$ -GT-SL Substrate Reagent (R2).

### REAGENT STABILITY AND STORAGE

The reagents included are stable until the expiry date stated on the labels at 2-8°C. The working reagent is stable for 30 days at 2-8°C.

### REAGENT DETERIORATION

The reagent solutions should be clear. Turbidity would indicate deterioration.

### INSTRUMENTS

Any instrument with temperature control of  $\pm 0.5^\circ\text{C}$  that is 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.

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## **SPECIMEN COLLECTION AND PREPARATION**

Fresh, clear, unhemolysed serum is the specimen of choice. Samples are stable (less than 10% change in activity) for 2 days at 18-26°C, 1 week at 0-4°C, and 1 month at -25°C (7).

## **INTERFERING SUBSTANCES**

It has been reported that some antiepileptic drugs (phenytoin, barbituates) produce false elevation of  $\gamma$ -GT levels (8).

Interferences from icterus, lipemia, and hemolysis were evaluated for this  $\gamma$ -GT method on a Hitachi analyzer using a significance criterion of >10% variance from control.

No significant icterus interference was found at bilirubin levels from 0-684  $\mu\text{mol/L}$  (0-40 mg/dL) in a 71 U/L  $\gamma$ -GT sample.

No significant lipemic interference was found at Intralipid levels from 0-1000 mg/dL (equivalent to 0-33.9 mmol/L [0-3000 mg/dL] triglycerides) in a 64 U/L  $\gamma$ -GT sample.

Hemoglobin levels of 0-155  $\mu\text{mol/L}$  (0-1000 mg/dL) were studied with acceptable results to 31  $\mu\text{mol/L}$  (200 mg/dL). At a hemoglobin level of 31  $\mu\text{mol/L}$  (200 mg/dL), a 10% negative interference was observed in a 64 U/L  $\gamma$ -GT sample.

A summary of the influence of drugs on clinical laboratory tests may be found by consulting Young, D.S. (9).

## **PROCEDURE**

### **Materials Provided**

The reagents necessary for the determination of  $\gamma$ -Glutamyltransferase are provided.

### **Materials Required**

1. An instrument which meets the requirements stated in the Instruments Section.
2. 1 cm cuvettes or a flow cell capable of transmitting light at 405 nm.
3. Test tubes of the appropriate size.
4. Pipettes of the appropriate size.
5. An appropriate timer.
6. An appropriate water bath.

### **Conditions**

|                         | Generic           | Automated Analyzer                           |
|-------------------------|-------------------|--|
| Wavelength              | 405 nm            | 405 nm                                       |
| Temperature             | 37°C              | 37°C   |
| Pathlength              | 1 cm              |  |
| Mode                    | Kinetic           | Kinetic                                      |
| Reaction Time           | 5 minutes         | 10 minutes                                   |
| Sample Volume           | 100 $\mu\text{L}$ | 10 $\mu\text{L}$                             |
| Reagent Volume          | 1.0 mL            | R1: 280 $\mu\text{L}$ , R2: 70 $\mu\text{L}$ |
| Total Volume            | 1.1 mL            | 350 $\mu\text{L}$                            |
| Sample to Reagent Ratio | 1:10              | 1:35   |

### **Procedure**

1. Prepare the required volume of working reagent as described under Reagent Preparation.
2. Into separate test tubes, pipette 100  $\mu\text{L}$  of serum to be assayed.
3. Add 1.0 mL of reagent, mix, and incubate for 2 minutes at 37°C.
4. Record the change in absorbance at 405 nm at one-minute intervals until the absorbance change is constant. The lag time will be reduced if the reagent is prewarmed to the incubation temperature.

## **CALIBRATION**

A standard is not used to calibrate the  $\gamma$ -GT procedure. Results are calculated using the molar extinction coefficient and the given formula (See Calculation and Results).

## **QUALITY CONTROL**

A normal and abnormal level control serum should be analyzed with each run of samples and the results should fall within plus or minus two standard deviations of the established value.

## **CALCULATION AND RESULTS**

### **Results**

$\gamma$ -GT activity is expressed as units per liter (U/L).

### **Calculation**

$$\begin{aligned}\gamma\text{-GT U/L} &= \frac{\Delta A/\text{min.} \times \text{assay volume (mL)} \times 1000}{9.5 \times \text{light path (cm)} \times \text{sample volume (mL)}} \\ &= \Delta A/\text{min.} \times 1158\end{aligned}$$

$\Delta A/\text{min.}$  = change in absorbance per minute  
Assay volume = total reaction volume expressed in mL  
1000 = converts U/mL to U/L  
9.5 = absorbance coefficient of 5-amino-2-nitrobenzoate at 405 nm  
Light path = length of the light path expressed in cm (usually 1.0)  
Sample volume = sample volume expressed in mL  
1158 = factor derived from the constants in the equation

### **Example**

$$\begin{aligned}\gamma\text{-GT U/L} &= \frac{0.041 \times 1.1 \times 1000}{9.5 \times 1 \times 0.1} \\ &= 0.041 \times 1158 \\ &= 47.5 \text{ U/L}\end{aligned}$$

0.041 = change in absorbance per minute  
1.1 = total reaction volume expressed in mL  
1000 = converts U/mL to U/L  
9.5 = absorbance coefficient of 5-amino-2-nitrobenzoate at 405 nm  
1 = length of light path expressed in cm  
0.1 = sample volume expressed in mL  
1158 = factor derived from constants in the equation

### **Limitations**

A sample with  $\gamma$ -GT level exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.

### **EXPECTED VALUES (4)**

Females: 4-25 U/L at 30°C                      5-32 U/L at 37°C  
Males: 7-40 U/L at 30°C                      9-52 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.

### **PERFORMANCE CHARACTERISTICS**

These performance characteristics were generated in DCL laboratories using automated procedures unless otherwise stated.

#### **Recovery Study:**

$\gamma$ -glutamyltransferase was added to pooled human sera to increase the  $\gamma$ -GT concentration by 78 U/L and 156 U/L. Recovery of the added  $\gamma$ -GT averaged 102.3%.

#### **Reportable Range (NCCLS EP6-P)**

Reportable range using automated procedures will depend on the sample to reagent ratio used. The automated procedure described gives a reportable range from 5-1000 U/L.

Precision Studies (NCCLS EP5-T2)

Data was collected on two control sera using a single lot of reagent in 40 runs conducted over 20 days.

| Level U/L | Total SD | Total CV % | Within Run SD U/L | Within Run CV % |
|-----------|----------|------------|-------------------|-----------------|
| 23        | 0.6      | 2.6        | 0.4               | 1.8             |
| 71        | 1.1      | 1.6        | 0.3               | 0.5             |

Accuracy

The performance of this method (y) was compared with the performance of a similar method (x) on a Hitachi analyzer. Forty-eight patient serum samples ranging from 20-236 U/L gave a correlation coefficient of 0.9993. Linear regression analysis gave the following equation:

$$\text{This method} = 1.03 (\text{reference method}) - 1.4 \text{ U/L.}$$

REFERENCES

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