GGT BR CE

REF 1126005	REF 1126010
2 x 50 mL	3 x 100 mL
CONTENTS	**CONTENTS**
R1. Reagent 2 x 40 mL	R1. Reagent 3 x 80 mL
R2. Reagent 1 x 20 mL	R2. Reagent 1 x 60 mL
For professional *in vitro* diagnostic use only	

γ-GT BR

IFCC

KINETIC

Quantitative determination of γ–GT activity

PRINCIPLE

Gamma-glutamyltransferase (γ-GT) catalyzes the transfer of a γ-glutamyl group from γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine with the formation of L-γ-glutamyl-glycylglycine and 5-amino-2-nitro-benzoate.
The amount of 5-amino-2-nitro-benzoate formed is proportional to the enzyme activity present in the sample monitored kinetically at 405 nm.[1]

(L-γ-glutamyl) -3-carboxy-4-nitroanilide + Glycylglycine $\xrightarrow{\gamma\text{-GT}}$ (L-γ-glutamyl) -glycylglycine + 5-amino-2- nitro-benzoate

This test has been formulated according the standardized method described by IFCC. Clin Chem Lab Med; 40(7) : 734-738.(2002)

REAGENT COMPOSITION

R1 **Buffer/Glycylglycine**. TRIS 133 mmol/L pH 8.2, glycylglycine 138 mmol/L, sodium azide 0.09%.

R2 **Substrate/Glupa-C**. L-γ-glutamyl-3-carboxy-4-nitroanilide 23 mmol/L, ethylene glycol 900 mmol/L, sodium azide 0.09%.

Warnings and precautions:

Warning.

Reagent 1. H319 - H335
P264 – 280 – P305 + P351 + P338 – P337 + P313 – P261 – P271 – P304 + P340 – P312 – P403 + P233 – P405 - P501
Reagent 2. H302
P264 - P270 – P301 + P312 – P330 - P501

Refer to the safety data sheets (SDS) available for download from our website www.linear.es and take the necessary precautions for the use of laboratory reagents.

STORAGE AND STABILITY

Store at 2-8ºC.
All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed, protected from light and prevented contaminations during the use.
Discard If appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 405 nm > 1.400 in 1cm cuvette.

REAGENT PREPARATION

Working reagent. Mix 4 mL of **R1** + 1 mL of **R2**. Stable for 3 weeks at 2-8ºC or for 5 days at 15-25ºC.
Protect from light.

SAMPLES

Serum or EDTA plasma free of hemolysis. Fluoride, citrate and oxalate inhibit γ-GT activity[2].
The enzyme in the sample is stable for at least 1 week at 2-8ºC and for at least 2 months when frozen.
Only freeze once. Discard contaminated specimens.
The samples should be handled as potentially infectious in accordance with Good Manufacturing Practices.

INTERFERENCES

- Lipemia (intralipid >2.5 g/L) may affect the results.
- Bilirubin (> 10 mg/dL) may affect the results.
- Hemoglobin (> 8 g/L) may affect the results.
- Other drugs and substances may interfere[6].

MATERIALS REQUIRED

- Photometer or spectrophotometer with cuvette thermostatable compartment set at 25/30/37ºC, capable of reading at 405 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.
- Ref. 1975005 Human Multicalibrator (optional).
- General laboratory equipment.

PROCEDURE

1. Preincubate working reagent and cuvettes to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

Working reagent	1.0 mL
Sample / Calibrator (optional)	100 µL

4. Mix and insert cuvette into the thermostatted cuvette holder and start the chronometer.
5. Incubate for 1 minute and record initial absorbance reading.
6. Make new readings every 60 seconds during 3 minutes.
7. Calculate the difference between absorbances.
8. Calculate the mean of the results to obtain the average absorbance change per minute (ΔA/min).

Calibration

With factor. The use of theorical factor is recommended to calculate the activity.

With calibrator. It is optional, it is only necessary if you do not apply the factor. Calibration must be performed with two points (S1: distilled water and S2: Calibrator). Verify the working reagent blank every day before its use.

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CALCULATIONS

With factor.

U/L **=** ΔA/min x **1230**

With calibrator.

$$\frac{\Delta A/min\ Sample}{\Delta A/min\ Calibrator} \times \text{Conc. Calibrator} = \text{U/L } (\gamma\text{-GT})$$

Samples with ΔA/min exceeding 0.200 at 405 nm should be diluted 1:10 with saline and assayed again. Multiply the results by 10.

If results are to be expressed as SI units apply:
U/L x 16.67 = nkat/L

REFERENCE VALUES[4]

Serum, plasma

Temperature	37ºC	30ºC	25ºC
Men	10-50 U/L (167-834 nKat/L)	7-35 U/L (117-583 nKat/L)	5-25 U/L (83-417 nKat/L)
Women	8-35 U/L (133-583 nKat/L)	6-25 U/L (100-417 nKat/L)	6-25 U/L (100-417 nKat/L)

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of γ-GT. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of γ-GT. Assayed.

If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE

Gamma-glutamyl transferase is the most sensitive enzymatic indicator available of hepatobiliary disease. The higher increments are found in cases of *intrahepatic* or *posthepatic biliary obstruction,* being more sensitive than alkaline phosphatase when detecting *obstructive jaundice*, *colangitis*, and *cholecystitis*.

Elevated levels are noted in the sera of patients with *alcoholic cirrhosis* and from people who are heavy drinkers. The enzyme levels are important in detecting alcohol induced liver disease correlating well with the duration of the drug action.

ANALYTICAL PERFORMANCE

- ***Detection Limit:*** 5.34 U/L
- ***Linearity:*** Up to 800 U/L
- ***Precision:***

U/L	Within-run		Between-run	
Mean	57.1	161.6	57.1	161.6
SD	0.43	0.81	0.16	0.72
CV%	0.75	0.50	0.89	0.44
N	10	10	10	10

- ***Sensitivity:*** 0,75 mA/min/U/L γ-GT.
- ***Correlation:*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 50 r = 0.99 y = 0.989x – 6.320

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result but should integrate both clinical and laboratory data.
3. The Assay Procedure must be followed closely, failure to follow the procedure may lead to inaccurate results.
4. To preserve the good functionality of the kit, do not mix different lots neither reagent's remains.
5. Disposal: Used materials should be discarded according to local regulations.

REFERENCES

1. IFCC Primary Reference Procedures for the Measurement of catalytic activity Concentrations of Enzymes at 37ºC. part 6. reference procedure for the measurement of catalytic concentratin of g-glutamyltransferase. *J. Clin. Chem. Clin. Biochem./Since* **40**, (2002).
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3. Szasz, G., Rosenthal, P. & Fritzsche, W. Die 1-Glutamyl-Transpeptidase-Aktivität im Serum bei hepatobiliären Erkrankungen. *DMW - Dtsch. Medizinische Wochenschrift* **94**, 46–61 (1969).
4. Tietz. N.W. Fundamentals of Clinical Chemistry, p.940. W.B. saunders Co. Philadelphia, PA. (1987).
5. Friedman y Young. Effects of disease on clinical laboratory tests. 5th ed. AACC (Press 2000).
6. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.

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