γ-GT (GGT)

L-γ-Glutamyltransferase
MANUAL
RX MONZA

INTENDED USE

For the quantitative *in vitro* determination of γ-glutamyl transferase (γ-GT) in serum or plasma. This product is suitable for manual use and on the RX **monza** analyser.

Cat. No.			
GT 523	R1a.	Buffer/Glycylglycine	1 x 105 ml
10 x 10 ml	R1b.	Substrate	10 x 10 ml

GTIN: 05055273203479

CLINICAL SIGNIFICANCE[1]

Gamma-glutamyltransferase (γ-GT) in serum originates primarily from the hepatobiliary system. Therefore γ-GT is elevated in all forms of liver disease and has been shown to be more sensitive than alkaline phosphatase in detecting obstructive jaundice, cholangitis and cholecystitis. High levels of γ-GT are also seen in patients with primary or secondary liver cancer. Increased levels are also observed in cases of alcohol abuse and in alcoholic liver cirrhosis. In patients receiving anticonvulsant drugs such as phenytoin and phenobarbital, increased levels of the enzyme in serum may reflect induction of new enzyme activity and the toxic effects of alcohol and other drugs on the microsomal structures in liver cells. Although γ-GT is the most sensitive enzymatic indicator of hepatobiliary disease it can not be used to differentiate between different types of hepatobiliary disease. However, γ-GT can be used in combination with other biochemical markers to discriminate between different types of hepatobiliary disease.

PRINCIPLE[2]
COLORIMETRIC METHOD

The substrate L-γ-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted by γ-GT in the sample to 5-amino-2-nitrobenzoate which can be measured at 405 nm.

L-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine

$$\xrightarrow{\gamma\text{-GT}}$$

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

SAMPLE COLLECTION AND PREPARATION

Serum: use only non-haemolysed serum
Plasma: use only EDTA plasma that is free from haemolysis. Other anticoagulants interfere with this test.
γ-GT in serum and plasma is stable for 7 days at +2 to +8°C or for 3 months at -20°C.

REAGENT COMPOSITION

Contents			Concentration in the Test
R1a.	**Buffer/Glycylglycine**		
	Tris buffer		100 mmol/l, pH 8.25
	Glycylglycine		100 mmol/l
R1b.	**Substrate**		
	L-γ-glutamyl-3-carboxy-4-nitroanilide		2.9 mmol/l

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solution R1a and R1b contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Health and Safety Data Sheets are available on request.

Please dispose of all biological and chemical materials according to local guidelines.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1a. Buffer/Glycylglycine
Contents stable as supplied up to the expiry date when stored at +2 to +8°C.

R1b. Substrate
Reconstitute one vial of Substrate R1b with **10 ml** of Buffer/Glycylglycine R1a. Stable for 21 days at +2 to +8°C or 5 days at +15 to +25°C.

MATERIALS PROVIDED

Buffer/Glycylglycine
Substrate

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Assayed Multi-sera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532).
Calibration Serum Level 3 (Cat. No. CAL 2351).
Pipetting device to deliver 100 µl and 1 ml.
Timing device and water bath or heating block to maintain temperature at +25°C, +30°C or +37°C.
Spectrophotometer with wavelength capability of 400 – 420 nm.

PROCEDURE

Aspirate fresh ddH_2O and perform a new Gain Calibration in flow cell mode. Select GGT in the Run Test screen and carry out a water blank as instructed.

Pipette into a test tube:	
Sample	0.05 ml
Reagent	0.50 ml

Mix and aspirate into the Rx Monza.

RX MONZA CALIBRATION

The use of Saline and Randox Calibration Serum Level 3 is recommended for calibration. Calibration is recommended with change in reagent lot or as indicated by quality control procedures.

STANDARDISATION

Randox Calibration Serum Level 3 is traceable to γ-GT reference material AD452 (IFCC) and JSCC TS01.

TEST METHOD FOR MANUAL USE

Wavelength:	Hg 405 nm (400 - 420 nm)
Cuvette:	1 cm light path
Temperature:	+25°C, +30°C, +37°C
Measurement:	against air

Pipette into cuvette:

Sample	0.10 ml
Reagent (+25°C, +30°C, +37°C)	1.00 ml

Mix, read initial absorbance and start timer simultaneously. Read again after 1, 2 and 3 minutes.

MANUAL CALCULATION

To calculate the GGT activity, use the following formula.

U/L = *1158 x –A 405 nm/minute

*Factor should be determined using the Randox Calibration Serum Level 3 for each new batch of reagent
Factor = ((Cal ABS - Blank ABS)/Cal Target)

QUALITY CONTROL

Randox Assayed Multi-sera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services, Northern Ireland + 44 (0) 28 9445 1070.

INTERFERENCE

Avoid haemolysis as it interferes with the assay.

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances[3]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

NORMAL VALUES IN SERUM[4]

	+25°C	+30°C	+37°C
Men	6-28 U/l	8-38 U/l	11-50 U/l
Women	4-18 U/l	5-25 U/l	7-32 U/l

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance characteristics were obtained using an RX **monza** running at +37°C with the aspiration volume set at 500 µl.

LINEARITY

The method is linear to 651 U/l. Dilute samples above this concentration 1+5 with 0.9% (w/v) NaCl solution and reassay. Multiply the result by 6.

SENSITIVITY

The minimum detectable level of γ-GT with an acceptable level of precision is 20.1 U/l.

PRECISION

Within Run

	Level 2	Level 3
Mean (U/l)	56.6	184
SD	1.89	3.56
CV (%)	3.3	1.93
n	20	20

Between Run

	Level 2	Level 3
Mean (U/l)	56.6	184
SD	4.26	4.29
CV (%)	7.5	2.33
n	20	20

METHOD COMPARISON

The Randox method (Y) was compared to another commercially available method (X). 41 patient samples, with values spanning the range 25 to 642 U/l were tested. Linear regression analysis of the data resulted in the following equation:

Y = 0.927X + 2.94
with a correlation coefficient of r = 0.9928

REFERENCES

1. Teitz, N. N., Fundamentals of Clinical Chemistry ed 3 Philadelphia, W B Saunders Co. 1987, pg 391.
2. Szasz, G., Clin Chem. 1969; **22**: 124-136.
3. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.
4. Szasz, G., Bergmeyer H.U., ed. Method of Enzymatic analysis. Weinheim Verlag Chemie, 1974.

The presence of a vertical bar in the margin indicates a technical update from the previous revision.

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