

Gamma GT Reagent

PRODUCT SUMMARY

Stability	:	21 days at 2-8°C
Linear Range	:	Up to 600 U/L (10.0 µkat/L)
Specimen Type	:	Serum or plasma
Method	:	Kinetic
Reagent Preparation	:	Add specified volume of distilled or deionized water.

IVD

INTENDED USE

This reagent is intended for the in vitro quantitative determination gamma-glutamyltransferase (GGT) [(γ-Glutamyl) - Peptide: Amino Acid γ-Glutamyltransferase, EC2.3.2.2], in human serum or plasma.

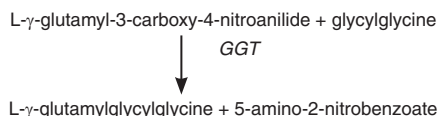
CLINICAL SIGNIFICANCE

Although GGT is present in a variety of tissues, the serum enzyme appears to be primarily from the hepato-biliary system. Consequently, GGT is elevated in all forms of liver disease or damage. It is clinically useful in detecting obstructive jaundice, cholangitis, and cholecystitis.

Elevated levels are also observed with drug use (alcohol, sedatives, anticonvulsants and tranquilizers).¹

METHODOLOGY

The first commercially available kinetic methods for the determination of GGT were based on the work of Szasz², Rosalki and Tarlow³. These methods utilised γ-glutamyl-p-nitroanilide (Glu-4-NA) as the substrate, however the poor solubility and stability of Glu-4-NA was a major limitation. In order to improve the method Persijn⁴ investigated further with Glu-4-NA derivatives and found that γ-glutamyl-3-carboxy-4-nitroanilide (Glucana) was superior to the Glu-4-NA with respect to both solubility and stability. The Glucana substrate now forms the basis of the IFCC and ECCLS recommended procedures. The GGT- Soluble Substrate method utilises Glucana in a one step method in which the reaction is initiated with the addition of sample. GGT present in the sample catalyzes the transfer of the glutamyl group from the substrate to glycylglycine forming glutamylglycylglycine and 5-amino-2-nitrobenzoate.



The rate of formation of 5-amino-2-nitrobenzoate is proportional to the activity of GGT present in the sample and can be measured kinetically at 405nm.

REAGENT COMPOSITION

Active Ingredients

	Concentration
Tris buffer	110 mmol/L
Glycylglycine	110 mmol/L
L-γ-glutamyl-3-carboxy-4-nitroanilide	3.2 mmol/L

Also contains non-reactive fillers and stabilizers

pH 8.20 ± 0.1 at 20°C.

WARNING: Do not ingest or inhale vapours. Avoid contact with skin, eyes and mucous membranes. If spilt thoroughly wash affected areas with water. Reagent contains Sodium Azide which may react with copper and lead plumbing. Flush with plenty of water when disposing. For further information consult the Gamma GT Reagent Material Safety Data Sheet. The Packaging of This Product Contains Dry Natural Rubber. Exercise precaution when handling metal crimps and broken glass vials, as sharp edges can injure the user.

R22 Harmful if swallowed.

R36/38 Irritating to eyes and skin.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

REAGENT PREPARATION

Reconstitute the contents of each vial with the volume of distilled or deionized water indicated on the vial label.

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use		Xn - Harmful

STABILITY AND STORAGE

Prior to reconstitution, when stored at 2-8°C and protected from light, the reagent is stable until the expiration date stated on the label. After reconstitution, the reagent is stable for at least 21 days when stored at 2-8°C or for 5 days at room temperature (18-25°C).

Indications of Reagent Deterioration:

- Turbidity;
- Failure to recover control values within the assigned range; and/or
- Reagent Absorbance >0.7 AU at 405nm (1cm lightpath).

SPECIMEN COLLECTION AND HANDLING

Serum: Use non-haemolysed serum.

Plasma: Heparin and EDTA only.

Storage: GGT is stable for 7 days when stored at 2-8°C.²

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

The equipment and material required for this procedure is:

- Clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 405nm.
- Distilled or deionized water for reagent preparation and related equipment e.g. pipettes.
- Analyser specific consumables, eg: sample cups.
- Normal and Abnormal assayed control material.

ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	25/30/37°C
Wavelength	405 nm (405 - 420 nm)
Secondary Wavelength	660 nm (600 - 660 nm)
Assay Type	Rate/Kinetic
Direction	Increase
Sample : Reagent Ratio	1 : 20
eg: Sample Vol	10 µL
Reagent Vol	200 µL
Lag Time	0-30 seconds
Read Time	3 minutes
Reagent Blank Limits	Low 0.0 AU
(405nm, 1cm lightpath)	High 2.0 AU
Linearity	Up to 600 U/L
(refer to linearity section)	(10.0 µkat/L)
Sensitivity	0.45 ΔmA/min per U/L
(405nm, 1cm lightpath)	(0.027 ΔA/min per µkat/L)

CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

Activity in U/L = ΔAbs/min x Factor

$$\text{Factor} = \frac{\text{TV} \times 1000}{9.5 \times \text{SV} \times \text{P}}$$

Where

TV = Total reaction volume in mL

SV = Sample volume in mL

9.5 = millimolar absorption coefficient of 5-amino-2-nitrobenzene at 405nm

P = Cuvette pathlength in cm.

Example:

$\Delta\text{Abs}/\text{min} = 0.117$
 Factor = 2210.5
 $\text{GGT} = 0.117 \times 2210.5 = 259 \text{ U/L}$

NOTES

- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- If the change in absorbance is greater than 0.52/min repeat the assay with less sample or sample diluted in 0.9% NaCl solution. Remember to adjust the factor for the smaller sample volume or to multiply the final result by the dilution factor.
- Unit Conversion:
 $\text{U/L} \times 16.67 \times 10^{-3} = \mu\text{kat/L}$.
- With most samples no lag phase is observed.

CALIBRATION

Not required. The rate of reaction is converted to U/L of activity by a calculation factor. Refer to the calculation section of this package insert.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least every eight hours or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling above the upper limit or below the lower limit of the established range indicates the assay may be out of control.

The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

- Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out on an automated clinical chemistry analyser. The following results were obtained:
Haemoglobin: No interference from haemoglobin up to 350 mg/dL.
Free Bilirubin: No interference from free bilirubin up to 820 $\mu\text{mol/L}$ (48 mg/dL).
Conjugated Bilirubin: No interference from conjugated bilirubin up to 1026 $\mu\text{mol/L}$ (60 mg/dL).
Lipaemia: No interference from lipaemia, measured as triglycerides up to 2.28 mmol/L (200 mg/dL).
- Young DS⁵ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES³

At 37°C : Males: < 50 U/L (0.83 $\mu\text{kat/L}$)
 Females: < 30 U/L (0.50 $\mu\text{kat/L}$)

 At 30°C*: Males: < 39 U/L (0.65 $\mu\text{kat/L}$)
 Females: < 23 U/L (0.38 $\mu\text{kat/L}$)

 At 25°C*: Males: < 28 U/L (0.47 $\mu\text{kat/L}$)
 Females: < 17 U/L (0.28 $\mu\text{kat/L}$)

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.

*Calculated results using temperature conversion factors of 0.77 for 30°C and 0.56 for 25°C. Thermo does not recommend the routine use of temperature conversion factors.

PERFORMANCE DATA

The following performance data was obtained with GGT reagent on an automated clinical chemistry analyser.

IMPRECISION

Within run	LEVEL I	LEVEL II
Number of data points	20	20
Mean (U/L / $\mu\text{kat/L}$)	20 / 0.334	67 / 1.12
SD (U/L / $\mu\text{kat/L}$)	0.41 / 0.007	1.00 / 0.017
CV%	2.05	1.49

Between Day

Between Day	LEVEL I	LEVEL II
Number of data points	20	20
Mean(U/L / $\mu\text{kat/L}$)	22 / 0.367	67 / 1.12
SD (U/L / $\mu\text{kat/L}$)	1.80 / 0.030	2.05 / 0.034
CV%	8.18	3.06

ACCURACY

Comparison studies were carried out using another similar commercially available GGT reagent. Serum and plasma samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:

Number of samples	147
Range of sample results	2 to 831 U/L (0.033 - 13.9 $\mu\text{kat/L}$)
Mean of reference method results	79 U/L (1.32 $\mu\text{kat/L}$)
Mean of GGT results	81 U/L (1.35 $\mu\text{kat/L}$)
Slope	0.99
Intercept	2.7 U/L (0.045 $\mu\text{kat/L}$)
Correlation Coefficient	0.999

LINEARITY

When run as recommended, the assay is linear up to 600 U/L (10.0 $\mu\text{kat/L}$).

SENSITIVITY

When run as recommended the sensitivity of this assay is 0.45 $\Delta\text{mA}/\text{min}$ per U/L (0.027 $\Delta\text{A}/\text{min}$ per $\mu\text{kat/L}$).

REFERENCES

- Kachmar JF, Moss DV. "Enzymes" in Fundamentals of Clinical Chemistry. Tietz NW (Ed) WB Saunders Co. Philadelphia 1976; page 621-3.
- Szasz G. Clin Chem 1969; 15: 124-36.
- Rosalki SB, Tarlow D. Clin Chem 1974; 20: 1121-4.
- Persijn JP and van der Slik W. J.Clin.Chem.Clin Biochem. 1976; 14: 421-7.
- Young DS. Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990; 3: 183-5.



Fisher Diagnostics
 a division of Fisher Scientific Company, LLC
 a part of Thermo Fisher Scientific Inc.
 Middletown, VA 22645-1905 USA
 Phone: 800-528-0494
 540-869-3200
 Fax: 540-869-8132



MDCI Ltd.
 Arundel House
 1 Liverpool Gardens
 Worthing, West Sussex BN11 1SL UK



840340 (R1)

© 2009 Thermo Fisher Scientific Inc. All rights reserved.

REF

Reorder Information

Catalogue No.	Configuration
TR19110	20 x 10 mL
TR19115	20 x 20 mL
TR19103	10 x 50 mL
TR19104	10 x 200 mL