

Gamma GT Reagent

2 Part Liquid

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Linear Range	:	Up to 1200 U/L (20.0 µkat/L)
Specimen Type	:	Serum
Method	:	Kinetic
Reagent Preparation	:	Supplied ready to use.

IVD

INTENDED USE

This reagent is intended for the in vitro quantitative determination of γ gammaglutamyltransferase (GGT) [(γ -Glutamyl) - Peptide: Amino Acid γ -Glutamyltransferase, EC2.3.2.2], in human serum on both manual and automated systems.

CLINICAL SIGNIFICANCE

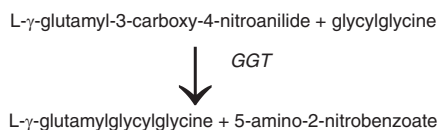
Although GGT is present in a variety of tissues, the serum enzyme appears to be primarily from the hepato-biliary system. Consequently, GGT is elevated in all forms of liver disease or damage. It is clinically useful in detecting obstructive jaundice, cholangitis, and cholecystitis. Elevated levels are 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- 2 Part Liquid method utilises Glucana in the following reaction, which is initiated by the addition of sample. GGT present in the sample catalyses 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
Reagent 1:	
Glycylglycine	130 mmol/L
Sodium Chloride	65 mmol/L
Also contains non-reactive fillers and stabilizers	
Reagent 2:	
L- γ -glutamyl-3-carboxy-4-nitroanilide	20 mmol/L

pH 8.15 \pm 0.1 at 20°C.

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt thoroughly wash affected areas with water. Reagent contains Sodium Azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the GGT 2 part Liquid Reagent Material Safety Data Sheet.

REAGENT PREPARATION

Reagents are supplied ready to use.

STABILITY AND STORAGE

Prior to use:

When stored between 2-8°C the reagents are stable until the expiration date stated on the bottle and kit box 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	REAG 1	Reagent 1 (R1)
REAG 2	Reagent 2 (R2)		

Once the Reagent is Opened:

When stored capped at 2-8°C, the reagents are stable until the expiration date stated on the bottle and kit box label. Combined reagent is stable for at least 6 weeks when stored at 2-8°C.

Indications of Reagent Deterioration:

- Turbidity;
- Reagent Absorbance >1.2 AU at 405nm (1cm lightpath); and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING

Serum: Use non-haemolysed serum.

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

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 405 nm.
- Analyser specific consumables, eg: sample cups.
- Assayed normal and abnormal control material.

ASSAY PROCEDURE

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

SYSTEM PARAMETERS

Temperature	30/37°C
Wavelength	405 nm (405-420 nm)
Secondary Wavelength	660nm (600 - 660nm)
Assay Type	Rate/Kinetic
Direction	Increase
Sample : Reagent Ratio	1 : 32 (R1): 8 (R2)
eg: Sample Vol	5 µL
Reagent 1 Vol	160 µL
Reagent 2 Vol	40 µL
Delay Time (sample + R1)	\leq 5 minutes
Lag Time (sample + R1 + R2)	> 60 seconds
Read Time	3 - 4 minutes
Reagent Blank Limits	Low 0.0 AU
(405nm, 1cm lightpath)	High 1.2 AU
Linearity	Up to 1200 U/L
(refer to linearity section)	(20.0 µkat/L)
Analytical Sensitivity	0.23 Δ mA/min per U/L
(405nm, 1cm lightpath)	(13.8 Δ mA/min per µkat/L)

CALCULATIONS

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

Activity in U/L = Δ Abs/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:

Δ Abs/min = 0.046

Factor = 4316

GGT = 0.046 x 4316 = 199 U/L

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. If the change in absorbance is greater than 0.27AU/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.
3. Unit Conversion: U/L x 16.67 x 10⁻³ = µkat/L.
4. 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.
- 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

1. Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out. The following results were obtained:

Haemoglobin: No interference from haemoglobin up to 59 µmol/L (500 mg/dL).

Unconjugated Bilirubin: No interference from bilirubin up to 275 µmol/L (16.3 mg/dL).

Conjugated Bilirubin: No interference from bilirubin up to 290 µmol/L (17.1 mg/dL).

Lipaemia: When measured bichromatically no interference from lipaemia, measured as triglycerides, up to 5.6 mmol/L (500 mg/dL).

2. 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 (0.835 µkat/L)
Females: < 30 U/L (0.501 µkat/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.⁶

PERFORMANCE DATA

The following performance data was obtained using the GGT 2 part Liquid reagent on an automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure.⁷

Within Run	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	26	95
Mean (µkat/L)	0.434	1.59
SD (U/L)	0.51	0.96
SD (µkat/L)	0.008	0.016
CV (%)	1.9	1.0

Total	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	26	95
Mean (µkat/L)	0.434	1.59
SD (U/L)	1.33	2.58
SD (µkat/L)	0.022	0.043
CV (%)	5.0	2.7

METHOD COMPARISON

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

Number of samples	62
Range of sample results	8 to 700 U/L (0.134 - 11.7 µkat/L)
Mean of reference method results	70 U/L (1.17 µkat/L)
Mean of GGT results	72 U/L (1.20 µkat/L)
Slope	1.05
Intercept	-0.88 U/L (-0.015 µkat/L)
Correlation Coefficient	0.999

LINEARITY

When run as recommended, the assay is linear up to 1200 U/L (20.0 µkat/L).


ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of this assay is 0.23 ΔmAbs/min per U/L (13.8 ΔmA/min per µkat/L).

REFERENCES

1. Kachmar JF, Moss DV. "Enzymes" in Fundamentals of Clinical Chemistry. Tietz NW (Ed) WB Saunders Co. Philadelphia 1976; page 621-3.
2. Szasz G. Clin Chem 1969; 15: 124-36.
3. Rosalki SB, Tarlow D. Clin Chem 1974; 20: 1121-4.
4. Persijn JP and van der Slik W. J.Clin.Chem.Clin Biochem. 1976; 14: 421-7.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990; 3: 183-5.
6. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.
7. National Committee for Clinical Laboratory Standards. User evaluation of Precision Performance of Clinical Chemistry Devices. NCCLS; 1984, NCCLS Publication EP5-T.

© 2009 Thermo Fisher Scientific Inc. All rights reserved. Hitachi is a registered trademark of Roche Diagnostics, Indianapolis, IN 46250. I.Lab 600 is a registered trademark of Instrumentation Laboratory Company, Lexington, MA 02421. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

 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



840395 (R1)

REF

Reorder Information

Catalogue No.

	REAG 1	REAG 2
TR19320	1 x 125 mL	1 x 35 mL
TL19301 (ILab 600)	5 x 80 mL	5 x 20 mL
TY19301 (Hitachi)	4 x 50 mL	4 x 14 mL
7500-116A	4 x 500 mL	
7500-216A		2 x 250 mL