GAMMA GLUTAMYL TRANSFERASE (GGT)

Diagnostic reagent for determination of GGT concentration.
Liquid. Dual reagents. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Pack
MH-195	100 mL
MH-196	50 mL
VH-195	100 mL
VH-196	50 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of Gamma Glutamyl Transferase (GGT) in serum and plasma.

GENERAL INFORMATION

GGT (EC 2.3.2.2.2; γ-glutamyl-peptide:amino acid γ-glutamyltransferase; GGT) catalyzes the transfer of the γ-glutamyl group from peptides or compounds to an acceptor.[1] The γ-glutamyl acceptor can be the substrate itself or some amino acids, peptides or even water, in which case simple hydrolysis occurs. Glycylglycine is five times more efficient as an acceptor than glycine or tripeptide (gli-gli-gli-gli), but little is known about the optimal properties of the acceptor cosubstrate.[2]

Human GGT is synthesized as a single polypeptide with a glycoprotein structure and 569 amino acid residues that are not enzymatically active. Activation occurs by a post-translational autoseparation reaction catalyzed by Threonine 381 residue.[2] The mature enzyme consists of two subunits with a molecular weight of 68 kDa, a 46-kDa subunit responsible for enzyme attachment on cellular membranes via a hydrophobic transmembrane domain and a 22-kDa subunit carrying the catalytic center.[3] The active GGT enzyme is encoded by the GGT1 gene on chromosome 22; alcohol is a known inducer of GGT gene expression.[2]

In order of frequency, GGT is found in the proximal renal tubule, liver, pancreas and intestine. The enzyme is found in the cytoplasm (microsomes), but a larger fraction binds to the outer surface of plasma membranes and can transport amino acids and peptides across the cell membrane into the cell in the form of γ-glutamyl peptides. GGT is critical for maintaining adequate intracellular concentrations of reduced glutathione, an important antioxidant agent.

In addition, GGT is involved in the metabolism of leukotrienes, xenobiotics and neurotransmitters (conversion of Gamma-Glutamyl-Taurine to Taurine) and in the modulation of nitric oxide signaling.[2]

Although renal tissue has the highest concentration of GGT, the enzyme in serum is primarily derived from the hepatobiliary system. The enzyme in serum is heterogeneous in both net molecular charge and size. These forms are composed of post-translational modifications of a uniform enzyme molecule rather than the presence of true isoenzymes.[2]

GGT is a sensitive indicator of the presence of hepatobiliary disease; it is increased in most people with liver disease regardless of cause, but its usefulness is limited by its non-specificity. Just like ALP, GGT activity is highest in cases of posthepatic biliary obstruction, reaching an activity approximately 10 to 30 times the upper reference limit (URL). High GGT increases have also been noted in patients with primary or metastatic liver neoplasms and other hepatic space-occupying lesions, possibly caused by intrahepatic obstruction.[2] Some studies suggest that GGT is a prognostic indicator in cases of hepatocellular carcinoma.[4] Recently, slightly elevated serum GGT has been found to be associated with cardiovascular disease and is being actively investigated as a marker of cardiovascular risk.[6] GGT actually accumulates in atherosclerotic plaques, suggesting a potential role in the pathogenesis of cardiovascular disease.[5]

Increases in GGT activity are observed in cases of drug-induced liver disease and in more than 50% of patients with non-alcoholic fatty liver disease, where the enzyme contributes to various diagnostic algorithms within the disease spectrum.[6] Moderate increases (two to five times the URL) occur in infectious hepatitis.

In pancreatitis and some pancreatic malignancies (especially if associated with hepatobiliary obstruction), enzyme activity may be 5 to 15 times the URL. Increased GGT activity is found in the serum of patients with alcohol-related liver disease [(aspartate aminotransferase/alanine aminotransferase ratio (AAR) >2] and in the serum of heavy drinkers; this increase may be used as a marker of hidden alcohol abuse. GGT also increases with higher body weight and obesity, and the effects of alcohol are more apparent in these groups.

Studies have also found increased enzyme concentrations in the serum of test subjects receiving anticonvulsant drugs such as phenytoin and phenobarbital. GGT activity is usually normal in acute MI. If there is an increase, it occurs on the fourth day and reaches a maximum value in the next 4 days, possibly indicating liver congestion due to right heart failure. Unlike ALP, serum GGT does not increase in the presence of increased osteoblastic activity, so measurement of the enzyme may be useful in distinguishing whether the source of increased ALP activity in serum is bone or liver.[2]

Epidemiologic evidence has shown that serum GGT activity has an independent prognostic value for cardiovascular morbidity and mortality.[7] In fact, experimental work has revealed that the active enzyme is present in atherosclerotic plaques and this seems to be related to the ability of GGT in mediating redox/prooxidation.[5] However, the addition of GGT to traditional risk factors is unlikely to significantly improve the prediction of cardiovascular events.[8]

TEST PRINCIPLE

Enzymatic colorimetric measurement

Szasz first published the first kinetic procedure for GGT in serum in the late 1960s using γ-glutamyl-p-nitroanilide as substrate and glycylglycine as acceptor.[9] Persijn and Van der Slik isr investigated various derivatives to overcome the poor solubility of γ-glutamyl-p-nitroanilide and found the substrate L-γ-glutamyl-3-carboxy-4-nitroanilide to be more stable and easily soluble in water.[10]

The substrate most commonly used in GGT measurements today is L-γ-glutamyl-3-carboxy-4-nitroanilide. In the 2002 IFCC reference measurement procedure for GGT, L-γ-glutamyl-3-carboxy-4-nitroanilide acts as the substrate, while glycylglycine acts as an acceptor. Buffering is provided by glycylglycine itself. The temperature of the reaction is 37 °C and the measurement wavelength of the reaction product (5-amino-2-nitrobenzoate) is 405 nm.[11]

In the measurement method, γ-glutamyltransferase transfers the γ-glutamyl group of L-γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine. The amount of 5-amino-2-nitrobenzoate formed at the end of the reaction is proportional to the GGT activity in the sample.
Determined by photometric measurement of the increase in absorbance at 405 nm wavelength.

REAGENT COMPONENTS

Reagent 1

Tris buffer	: <500 mol/L
Glisilglisin	: <500 mol/L
Sodium azide	: <%0.1

Reagent 2

L-γ-glutamil-3-karboksi-4-nitroanilit	:< 25 mmol/L
Sodium azide	: <%0.1

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 60 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[12]

SAMPLE REQUIREMENTS

Serum and plasma are collected by standard procedure. Li-heparin and Na-heparin sample collection tubes should be preferred for plasma. Hemolysis should be avoided. Multiple sample freezing and thawing should be avoided.

GGT activity stability in serum and plasma[26,27]:

7 days at +2/+8ºC
7 days at +20/+25ºC
1 year at -20°C

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of Arcal Auto Calibrator.

Arcal Auto Calibrator-Lyophilized
Ref.No: VT-003

Calibration stability is 60 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Traceability is ensured with the ERM-AD452/IFCC sample.
Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N Level 1 Control- Lyophilized
Ref.No: VT-001

Arcon P Level 2 Control- Lyophilized
Ref.No: VT-002

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Serum/Plasma[28] ;
Men : < 60 U/L (< 1.00 µkat/L)
Women : < 40 U/L (< 0.67 µkat/L)

Annotation:

- Reference limits are approximately twice as high in people of African descent. In normal term newborns, GGT activity at birth is approximately six to seven times the adult reference interval.[13] Activity then decreases and reaches stable values by 6 months of age. However, sex-specific differences are only observed after the onset of puberty.[14]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[15]

Unit Conversion:
U/L × 0.0167 = µkat/L

PERFORMANCE CHARACTERISTICS

Measuring Interval
According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[16]

The determined analytic measuring interval for GGT is 4 – 500 U/L.

Detection Capability

Limit of Detection (LoD): 1 U/L
Limit of Quantitation (LoQ): 4 U/L

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[17]

Linearity

This method shows measurement linearity in the activities up to 500 U/L. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:10 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[18]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[19]

Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of GGT have been given in the table 1 and 2 respectively.

Table 1. GGT Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
27 U/L	0.30	1.11	80
168 U/L	0.88	0.52	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[20]

Table 2. GGT Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
27 U/L	0.68	2.51	80
168 U/L	2.11	1.25	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[20]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablock equation:[21]
y= 1.02x – 0.32 U/L
r= 0.997

Interference

Endogenous interferant and analyte concentrations that have been used in the GGT scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[22,23]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from GGT interference scanning test is appropriate, is determined as ±10%.[24]

In GGT test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	GGT Target (U/L)	N*	Observed Recovery %
Bilirubin 7,12 mg/dL	25,3	3	91

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[23]

Annotation:

- Some researchers have shown that fluoride, oxalate and citrate at a concentration of 1 g/L inhibit GGT by approximately 15%.
- Divalent ions such as calcium and magnesium and monovalent ions such as sodium and potassium have no effect on the catalytic activity.[25]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[23]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032 :Releases a very toxic gas if contacts with acid.
H317 :May cause allergic skin reaction.

Precaution

P280 :Use protective gloves / clothes / glasses / mask.
P264 :Wash your hands properly after using.
P272 :Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352 :Wash with plenty of water and soap if it contacts with skin.
P333+P313 :Seek medical help if it irritates your skin or develops rash.
P362+P364 :Remove contaminated clothes and wash properly before using.

Disposal

P501 :Dispose the vials and contents according to the local regulations.

REFERENCES

1. Goldberg DM. Structural, functional, and clinical aspects of gamma-glutamyltransferase. CRC Crit Rev Clin Lab Sci 1980;12:1–58.
2. Rifai, N., Chiu, R. W., & Young, I., et al., (2023) Tietz Textbook of Laboratory Medicine (7th ed.), Chapter 32: Serum Enzymes, p.350-350.e36, Elsevier, St. Louis, Missouri 63043
3. Whitfield JB. Gamma glutamyl transferase. CRC Crit Rev Clin Lab Sci 2001;38:263–355.
4. Xia J, Song P, Sun Z, Sawakami T, Jia M, Wang Z. Advances of diagnostic and mechanistic studies of g-glutamyl transpeptidase in hepatocellular carcinoma. Drug Discov Ther 2016;10:181–7.
5. Emdin M, Pompella A, Paolicchi A. Gamma-glutamyltransferase, atherosclerosis, and cardiovascular disease: triggering oxidative stress within the plaque. Circulation 2005;112:2078–80.
6. Dillon JF, Miller MH. Gamma glutamyl transferase 'To be or not to be' a liver function test? Ann Clin Biochem 2016;53: 629-31.
7. Bulusu S, Sharma M. What does serum g-glutamyltransferase tell us as a cardiometabolic risk marker? Ann Clin Biochem 2016;53:312-32.
8. Kunutsor SK, Bakker SJ, Kootstra-Ros JE, Gansevoort RT, Dullaart RP. Circulating gamma glutamyltransferase and prediction of cardiovascular disease. Atherosclerosis 2015;238:356-64.
9. Szasz G. A kinetic photometric method for serum γ-glutamyltransferase. J Clin Chem 1969;15:124-136.

10. Persijn JP, van der Slik W. A new Method for the Determination of γ-Glutamyltransferase. J Clin Chem Clin Biochem 1976;4:421.
11. Schumann G, Bonora R, Ceriotti F, Clerc-Renaud P, Ferard G, Ferrero CA, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 6. Reference procedure for the measurement of catalytic concentration of γ-glutamyltransferase. Clin Chem Lab Med 2002;40:734-8.
12. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI Document EP25-A. Wayne, PA: CLSI; 2009.
13. Cabrera-Abreu JC, Green A. g-Glutamyltransferase: value of its measurement in pediatrics. Ann Clin Biochem 2002;39:22–5.
14. Zierk J, Arzideh F, Rechenauer T, Haeckel R, Rascher W, Metzler M, Rauh M. Age- and sex-specific dynamics in 22 hematologic and biochemical analytes from birth to adolescence. Clin Chem 2015;61:964–73.
15. Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition. CLSI Document EP28-A3c. Wayne, PA: CLSI; 2010.
16. Clinical and Laboratory Standards Institute (CLSI). Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking – 1st Edition. CLSI Document EP34. Wayne, PA: CLSI; 2018.
17. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.
18. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach - 1st Edition. CLSI Document EP06-A. Wayne, PA: CLSI; 2003.
19. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.
20. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.
21. Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.
22. Clinical and Laboratory Standards Institute (CLSI). Supplemental Tables for Interference Testing in Clinical Chemistry - First Edition. CLSI Document EP37. Wayne, PA: CLSI; 2018.
23. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry - Third Edition. CLSI Document EP07. Wayne, PA: CLSI; 2018.
24. CLIA proficiency testing criteria for acceptable analytical performance, as printed in the Federal Register July 11, 2022;87(131:41194-242.
25. Pesce, A. J., & Kaplan, L. D. (2009). Methods in Clinical Chemistry: Kaplan and Pesce's: Clinical Chemistry: Theory, Analysis, Correlation: Vol. I (5th ed.), Chapter: Gammaglutamyl Transferase (GGT), p.609-17. Elseviers.
26. Szasz G. Methods of Enzymatic Analysis. 2nd English ed. New York. Academic Press, Inc 1974;717.
27. Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia PA: WB Saunders Company 1995;286
28. Thomas L, Müller M, Schumann G, et al. Consensus of DGKL and VDGH for interim reference intervals on enzymes in serum. J Lab Med 2005; 29(5):301-308.

Archem Sağlık Sanayi ve Tic. A.Ş. (With official contract based manufacturing agreement with Validity Sağlık Hiz. Sanayi A.Ş. Company)
Mahmutbey Mah. Halkalı Cad. No:124 Kat:4
Bağcılar/İstanbul/Turkey
Tel: + 90 212 444 08 92
Fax: +90 212 629 98 89
info@archem.com.tr www.archem.com.tr
info@validity.com.tr www.validity.com.tr

CE

SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests