MEDICA

IVD CE

REF 10219-4 4 x 29 mL / 7 mL

GAMMA-GLUTAMYL TRANSFERASE (GGT)

Wedges each contain usable volumes of 29 mL of R1 reagent and 7 mL of R2 reagent.

INTENDED USE

The EasyRA GGT reagent is intended for the quantitative determination of gamma-glutamyl transferase activity in human serum and plasma (with lithium heparin as anticoagulant), using the MEDICA EasyRA® Clinical Chemistry Analyzer.
For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION

Gamma-Glutamyl Transferase catalyzes the transfer of the γ-glutamyl group from a γ- glutamyl peptide to an amino acid of another peptide. The enzyme was first characterized and purified by Szewczuk and Baromouski.[1] The activity of GGT in the blood is used to diagnose liver diseases such as alcoholic cirrhosis and primary and secondary liver tumors.[2] Early serum measurement of γ–GGT involved the use of a relatively insoluble L-γ–glutamyl-P-nitroanilide substrate.[3, 4] Persijn and Van der Slik[5] modified the method by utilizing a more soluble and stable substrate — L-γ–glutamyl-3-carboxy-4-nitroanilide. The International Federation of Clinical Chemistry (IFCC) further modified this procedure for the measurement of GGT in serum.[6]

PRINCIPLE OF THE PROCEDURE

The substrate L-γ–glutamyl-3-carboxy-4-nitroanilide substrate (R2) transfers its glutamyl group to glycylglycine (R1) in the presence of GGT, forming 5-amino-2-nitrobenzoate + L-γ-glutamylglycylglycine.

L-γ–glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{\text{GGT}}$ 5-amino-2-nitrobenzoate + L-γ-glutamylglycylglycine

The 5-amino-2-nitrobenzoate product is measured spectrophotometrically at 405 nm. The rate of the formation of this component is directly proportional to the amount of Gamma-Glutamyl Transferase activity in the sample.

REAGENTS

GGT Buffer Reagent (R1):
L-glycylglycine 100 mmol/L
Buffer pH 8.3 (at 25°C) and a preservative.

GGT Substrate Reagent (R2):
L-γ–glutamyl-3-carboxy-4-nitroanilide 4 mmol/L

PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (*CLSI,* GP17-A2).
2. The reagents contain less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2 – 8°C. The reagent is stable on-board in the refrigerated reagent area of the EasyRA Analyzer for the number of days programmed on the RFID chip on the reagent wedge. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values.

SPECIMEN COLLECTION AND STORAGE / STABILITY

Use serum and plasma free from significant hemolysis. Lithium heparin coated tubes may be used for plasma collection. Serum and plasma samples without a preservative should be separated from cells and clots as soon as possible. Serum and plasma GGT are stable for 2 days at 18 – 25°C, 1 week at 2-8ºC and 1 month at -25°C.[7]

PROCEDURE

Materials Provided
Medica GGT Reagent Wedge, REF 10219-4

Additional materials required
Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use
The reagent is ready to use as supplied. Remove the cap and place the reagent in the EasyRA Analyzer reagent tray located in the reagent area. The on-board stability (60 days maximum) is programmed on the RFID chip on the reagent wedge.

Note: Check inside the necks of the wedge for foam after removing the caps and placing the wedge on the EasyRA Analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2.

Calibration
Not applicable.

Quality Control

It is recommended that two levels of human serum based controls (normal and abnormal) be run with the assay daily when patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results
After completion of the assay, the EasyRA Analyzer calculates the GGT concentration from the change in absorbance per minute, sample volume, total reaction volume, pathlength (cm) of 0.6 and molar absorptivity of 9.5.

$$\text{GGT (U/L)} = (\Delta\text{A/Min}) \times \frac{(\text{Total Volume}(\mu l) \times 1000)}{(\text{Molar absorptivity} \times \text{Pathlength(cm)} \times \text{Sample Volume}(\mu l))}$$

A unit per liter (U/L) of GGT activity is the amount of enzyme, which produces one μmol/L of NADH per minute.

Expected Values[7]
The reference range for GGT in serum and plasma is as follows:

Adult Male: 9 – 52 U/L (at 37°C)
Adult Female: 5 – 32 U/L (at 37°C)

These values are guidelines. It is recommended that each laboratory establish its own range of expected values since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g. if sample is above assay range)
If the Absorbance Change per Minute (ΔA/Min) is greater than 0.1357, which corresponds to approximately 1000 U/L, results will be flagged with “SD” (substrate depletion) by the analyzer. If the “Re-run” icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the GGT test to 2000 U/L.

PERFORMANCE CHARACTERISTICS[8]

Reportable Range
The reportable range is 7 to 1000 U/L. Extended range is 7 to 2000 U/L when half of the sample is used (1:1 dilution).

Inaccuracy/ Correlation (CLSI, EP9-A2)

The following table lists the data obtained in a comparison of the Medica Reagent for GGT (y) on the EasyRA Analyzer utilizing a primary wavelength of 405 nm only to the performance of the same GGT reagent (x) on the EasyRA Analyzer utilizing a primary wavelength of 405 nm and a secondary wavelength of 700 nm. The data shown below represents single determinations obtained utilizing a primary wavelength and a secondary wavelength on the EasyRA Analyzer vs. the average of two replicate values obtained utilizing a primary wavelength on the EasyRA Analyzer.

Number of Samples	47	Range of Samples	10 to 903 U/L
Slope	1.0213	y Intercept	-0.9888
Correlation Coefficient	0.9999	Regression Equation	Y = 1.0213*X-0.9888

The following table lists the data obtained in a comparison of matched serum (x) and Lithium heparinized plasma (y) samples using the Medica Reagent for GGT on the EasyRA Analyzer. The data below represents a single plasma determination vs. the average of two replicate serum values.

Number of Samples	80	Range of Samples	10 to 1057 U/L
Slope	1.0319	y Intercept	-3.8998
Correlation Coefficient	0.9997	Regression Equation	Y = 1.0319*X – 3.8998

Imprecision (CLSI, EP5-A2)
Duplicate measurements of each of three levels material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

Level U/L	Within Run SD U/L	Within Run CV %
88	1.0	1.2
61	1.0	1.7
34	0.9	2.5

Total Imprecision:

Level U/L	Total Imprecision SD U/L	Total Imprecision CV %
88	1.9	2.1
61	1.5	2.4
34	1.0	3.0

Linearity (CLSI, EP6-A)
Linear from 7 to 1000 U/L, based on the linear regression equation Y = 1.0126*X + 0.6421

Limit of Blank (LOB):	4.0 U/L	(CLSI, EP17-A)
Limit of Detection (LOD):	5.3 U/L	(CLSI, EP17-A)
Limit of Quantitation (LoQ):	6.6 U/L	(CLSI, EP17-A)

Interfering Substances (CLSI, EP7-A)
Less than 10% interference was classified as “no significant interference.”
Hemoglobin interferes, even in minimal concentrations. Do not use hemolyzed samples.
No significant interference was found with levels up to 35 mg/dL of total bilirubin.
No significant interference was found with levels up to 20 mg/dL of direct bilirubin.
No significant interference was found with levels up to 667 mg/dL of triglycerides (*using Intralipid***).

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC*.

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[9,10]

REFERENCES

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8. Data on file at Medica
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EasyRA Assay Parameters (GGT)

Primary Wavelength (nm)	405
Secondary Wavelength (nm)	700
Reaction Type	Enzyme (0)
Reaction Direction	Increase
Reagent Blank	No
Sample Blank	No
Max. first interval abs. Change	0.054
Reaction Time	7.2 min
Calibration interval (maximum)	N/A
Reagent on-board stability	60 days

Serum/Plasma

Sample volume (µl)	5.0
Diluent volume (µl)	40
Reagent volume R1 (µl)	132
Reagent volume R2 (µl)	33
Decimal Places (default)	0
Units (default values)	U/L
Dilution Factor	1:1 (to extend measuring range)
Linearity	7 to 1000 U/L
Molar Absorptivity	9.50

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