GGT-MK-1010 **(10x10 ml)** ***GGT-MK-0610*** **(6x10 ml)**
GGT-MK-0520 **(5x20ml)**

INTENDED USE

NS Biotec γ-GT Reagent is intended for the in vitro quantitative determination of γ-glutamyltransferase in serum and plasma on both automated and manual systems

CLINICAL SIGNIFICANCE

γ -glutamyltransferase (γ –GT) is a peptidase which hydrolytically cleaves glutamic acid, attached to the amino terminus of proteins or peptides through its γ –carboxyl group, and reconjugates it to a suitable acceptor (peptides, water, L-amino acids). γ –GT is important in glutathione metabolism. The highest concentration of γ –GT is found in the luminal membrane of the proximal tubules of the kidney. Other sources are the pancreas, prostate and liver. Clinically significant elevations of γ –GT present in serum are almost exclusively associated with hepatobiliary diseases. Use of γ –GT activity in the diagnosis of hepatic dysfunction appears to be much more sensitive than the use of other liver enzymes, since elevation of γ –GT occurs earlier and lasts longer. The highest elevations are found in intrahepatic or posthepatic biliary obstruction, where values may be 5 to 30 times of normal levels. Moderately elevated γ –GT levels are seen in hepatitis, cirrhosis, fatty liver disease states, metastatic hepatic neoplasm, and acute or chronic pancreatitis. Increased levels of γ –GT are also seen in sera of heavy drinkers or patients with alcohol cirrhosis. High γ –GT activity is found in prostate tissue, which may account for the increased γ –GT activity seen in some sera from men compared with sera from women.

ASSAY PRINCIPLE

γ -Glutamyltransferase catalyzes the transfer of a γ-glutamyl group from a substrate to an appropriate acceptor. Development of clinical methodologies has been concerned with selection of both acceptor and substrate compounds since both affect the sensitivity and convenience of the method. Methods have utilized γ-glutamylanilide or γ-glutamylnaphthylamide as the substrates. The liberated aromatic compound is measured by the Bratton-Marshall reaction. These methods are not continuous, however, and the product in one of these approaches, γ -naphthylamine, is carcinogenic. Szasz developed a kinetic approach in which Glupa-Carboxylat was used as the substrate; and glycylglycine, the acceptor. The use of glycylglycine accelerates the reaction greatly over the rate obtained in simple buffered medium. The series of reactions involved in the assay system is as follows:

1. NS Biotec reagent uses Glupa-Carboxylat as the donor substrate and glycylglycine as the acceptor substrate. Using these substrates, the following reaction is catalyzed by the presence of γ-GT.
2. Yellow colored product (5-amino-2-nitrobenzoate) is formed.

Glupa-Carboxylate +glycylglycine $\xleftrightarrow{\gamma GT}$ **g-Glutamyl-glycylglycine +5-amino-2-nitrobenzoate**

The rate of 5-amino-2-nitrobenzoate formation is directly proportional to the γ-GT activity in the specimen. It is determined by measuring the increase in absorbance at 405 nm.

EXPECTED VALUE

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the □□-GT results should always be assessed in conjunction with the patients medical history, clinical examination and other findings.

	Male	Female
25^{0}C	6 - 28 U/l 0.1- 0.5 µkat/l	4 - 18 U/l 0.07- 0.3 µkat/l
30^{0}C	8 – 38 U/l 0.1- 0.6 µkat/l	5 - 24 U/l 0.08 - 0.4 µkat/l
37^{0}C	11 - 50 U/l	7 - 32 U/l

REAGENTS

R_1	Tris buffer pH 8.2 glycylglycine	100 100	mmol/l mmol/l
R_2	Glupa-Carboxylate	2.9	mmol/l

- **Reagent Preparation & Stability**

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8^{0}C.

According to requirements, prepare the working solution by mixing 8 volumes of **R_1** and 2 volume of **R_2**. Mix well, do not shake, protect from direct light. the working solution is stable:

1 week at 20–25 ^{0}C.
6 weeks at 2 – 8 ^{0}C.

SPECIMEN

Serum or heparinized plasma.

Specimen Preparation & Stability

Freshly collected serum specimen should be kept at room temperature and assayed as soon as possible but not later than 4 hours after collection.

Complexing anticoagulants such as citrate, oxalate, and EDTA must be avoided. The γ-GT activity remains stable in serum samples for at least 5 days at 4^{0}C and for 9 months at –80^{0}C.

PROCEDURE

- **Manual Procedure**

Wavelength	405 nm
Cuvette	1 cm light path
Temperature	25, 30 or 37 ^{0}C
Zero adjustment	against air

Pipette into test tube or cuvette	
Working solution	1000 µl
Serum or plasma	100 µl

Mix, incubate for 60 seconds, and start stopwatch simultaneously. Read again after exactly 1, 2, and 3 minutes.

- ***Automated Procedure***

User defined parameters for different auto analyzers are available upon request.

CALCULATION

Determine the change in absorbance per minute (ΔA/min) from the linear portion of the reaction curve and calculate the γ-GT concentration by using the following formulae:

$$U/l = 1158 \times \Delta A \ 405\ nm/min$$

One international unit **(U)** is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

The general formula for converting ΔA/min into U/l is:

$$U/l = \frac{\Delta A/min \times TV \times 1000}{\sum \times SV \times LP}$$

Where:

TV	Total reaction volume in ml
SV	Sample volume in ml
*∑	millimolar absorptivity of

	Glupa Carboxylat
LP	Cuvette path length in cm
1000	Conversion of U/ml to U/l

- **Unit conversion**
  U/l x 16.67 x 10^{-3} = kat/l

- **Temperature correction**
  Multiply the result by 1.36 if the assay performed at 25^{0}C but is to be reported at 30^{0}C, and 1.78 if the assay performed at 25^{0}C but is to be reported at 37^{0}C.

LINEARITY

When run as recommended, the assay is linear up to 235 U/l or 3.9 µkat/l.

If result exceeds 235 U/l or 3.9 µkat/l, specimen should be diluted 1+5 with 0.9% NaCl solution and reassayed. Multiply the result by 6.

SENSITIVITY

The sensitivity is defined as the lower detection limit represents the lowest measurable γ-GT concentration that can be distinguished from zero.

When run as recommended the sensitivity of this assay is 1 U/l or 0.017 µkat/l

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established γ-GT values may be routinely used for quality control.

The assigned value of the control material must be confirmed by the chosen application.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

Control results falling outside the upper or lower limits of the established ranges indicate 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 NS Biotec Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**
  Complexing anticoagulants such as citrate, oxalate, and EDTA inhibit the enzyme activity. The only acceptable anticoagulant is heparin
- **Bilirubin:**
  No significant interference from free or conjugated bilirubin up to a level of 60 mg/dl.
  Drugs:
  Young[8] in 1990 has published a comprehensive list of drugs and substances which may interfere with this assay.
- **Haemolysis:**
  Haemoglobin levels higher than 500 mg/dl decrease the apparent γ-GT activity significantly.
- **Lipemia:**
  No significant interference. Lipemic specimens may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun

WARNING & PRECAUTIONS

NS Biotec γ-GT reagent is for in vitro diagnostic use. Normal precautions exercised in handling laboratory reagents should be followed.

- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- Don't use the reagent if it is turbid or if the absorbance against water is greater than 0.8 at 405 nm.

BIBLIOGRAPHY

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3. **Moss, DW, Henderson, AR, and Kachmar, JF (1987):** Enzymes. In: Tietz, NW, ed. Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders. 346-421.

	Consult Instruction for Use
	Caution Consult Accompanying Documents
IVD	In Vitro Diagnostic Medical Device
	Temperature Limitation
	Manufacturer
EC REP	Authorized Representative In The European Community
REF	Catalogue Number
LOT	Batch Code
	Use By

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