Manual Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Automated procedure on request

Cat. No. 13421	R1	1	x	50	ml
For 50 tests	R2	5	Powder for	10	ml

γ-GT

L-γ-Glutamyl-Transferase
Kinetic colorimetric method (DGKC)

Powder Reagent

Test Principle

L-γ-Glutamyl-3-carboxy-4-nitranilide + Glycylglycine $\xrightarrow{\gamma\text{-}GT}$ 5- Amino-2-nitrobenzoate +L-γ-Glutamyl-glycylglycine

L-γ-Glutamyltransferase in the sample catalyzes the transfer of the glutamyl group from L-γ-glutamyl-3-carboxy-4-nitroanilide (GLUPAC) to Glycylglycine according to the above reaction. The amount of 5-amino-2-nitrobenzoate formed is proportional to γ-GT activity which could be measured kinetically at 405 nm by measuring the increasing intensity of the yellow color formed.

Concentrations in the test

Reagent R1		
Tris-Buffer, pH = 8.25	100	mmol/L
Glycylglycine	100	mmol/L
Reagent R2		
L-γ-Glutamyl-3-carboxy-4-nitranilide	26	mmol/L

Stability and preparation of working reagent

Reagent R1: *liquid.*
Reagent R2: *powder.*
Avoid direct exposure to light.
All reagents are stable up to expiry date given on the label when stored at +2 ➔ +8 °C.

Working Reagent: Add 10 ml of bottle R1 into bottle R2 and mix gently. Stability: 2 months at 2 - 8 °C.

Note: Don't use if moisture has entered the vial and caking has occurred, or if the reconstituted reagent has an absorbance greater than 0.85 at 405 nm when read against water.

Specimen collection and handling

Serum, EDTA or Heparinized plasma, free from hemolysis.
Stability: 7 days at 2 - 8 °C.

Calibrator

MediCal U Cat. No. 15011

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 405 (400 – 415 nm)
Spectrophotometer	405 nm
Cuvette	1 cm light path
Temperature	37 °C
Measurement	against air or distilled water
reaction	Kinetic - increase

Assay: Incubate Working Reagent at 37°C before use:

Sample	100 µl
Working Reagent	1000 µl
Mix, incubate for 1 min. at 37 °C. Read change in the absorbance per 1 min. for 3 min. Determine the mean absorbance change per 1 min. (ΔA/min).	

Calculation

γ - GT activity (U/L) = ΔA/min × Factor

Factor = 1158

Note: It is recommended that each laboratory (as per instrument performance) could make its own factor (F) by the use of a calibrator according to the following formula:

$$F = \frac{Conc_{calibrator}}{\Delta A/\min_{Calibrator}}$$

Linearity

Up to 230 U/L.
If the result exceeds 230 U/L or If absorbance change (ΔA/min) exceeds 0.200, repeat the test using diluted sample (1+5) with sodium chloride solution (0.9 %) and multiply the result by 6.

Interferences

1. Anti-epileptic drugs (phenytoin and barbiturates) may falsely elevate γ-GT levels.
2. Bilirubin up to 20 mg/dl and hemoglobin up to 100 mg/dl have been found to have a negligible effect on this procedure.
3. For a comprehensive list of drug interference, see Young *et al*.

Precautions

1. Reagent R1 may be irritating to skin, flush skin with water if contacted.
2. Reagent R1 contains sodium azide as a preservative. Don't ingest. May react with lead and copper plumbing to form highly explosive metal azide. Upon disposal, flush with large volume of water to prevent azide build up.

Reference range

Newborn		< 185	U/L
Children		< 32	U/L
Adults	women	< 35	U/L
	men	< 40	U/L

References

1. Sasz,G., Persijn, j. P. et Al., z.Klin. Chem. and Klin. Biochem. 12 (1974) 228.
2. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.
3. Shaw LM, Stromme JH, London JL, Theodorsen L. LFCCmethod for y-glutamyltransferase. J Clin Chem Clin Biochem 1983, 21: 633 - 46.
4. German Society for Clinical Chemistry. Recommendations for carrying out standard ECCLS procedures (1988) for catalytic concentrations of creatine kinase, aspartate aminotransferase, alanine aminotransferase and y-glut-amyltransferase at 37 0C.
   Eur Clin Chem Clin Biochem 1993, 31: 901 - 9.
5. Persijn JP, v.d. SlikW. A new method for the deterniination of y-glutamyltransferase in serum. I Clin Chem Clin Biochem 1976, 14: 421 - 7.