γGT

Kinetic determination of γ-glutamyltransferase in serum and plasma

CE IVD

ORDER INFORMATION

REF	Kit size
GA4544 00	5x40 + 1x50 ml
KL4544 00	8x16 + 8x4 ml
BK4544 00	2x(80+20 ml)

INDICATION

γ GT (γ-glutamyltransferase) is an enzyme present in the kidney, pancreas, liver, and prostate. It is a sensitive indicator of liver disease, very helpful in diagnosing hepatobiliary obstruction, and is elevated in all forms of liver disease and alcoholism.

METHOD PRINCIPLE

Kinetic determination of γ-glutamyltransferase activity according to the following reaction:

$$\text{L-}\gamma\text{-glutamyl-3-carboxy-4-nitroanilide} + \text{glycilglycine} \xrightarrow{\gamma GT} \text{L-g-glutamylglycine} + \text{5-amino-2-nitrobenzoate}$$

COMPOSITION

REAGENT A:

Tris buffer, pH 8.25	100 mmol/l
Glycilglycine	100 mmol/l
Stabilizers	

REAGENT B:

L-γ-glutamyl-3-carboxy-p-nitroanilide	4 mmol/l
Stabilizers	
Harmful (x_n)	**R 22; S 36**

PREPARATION OF REAGENTS

Bireagent procedure:
The reagents are liquids ready to use.

Monoreagent procedure:
Mix 4 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 20 ml of RA + 5 ml of RB).

Storage and stability
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 30 days at 2-8 °C.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum, EDTA plasma.
Stable 7 days at 2-8 °C. Store at -20 °C for longer period.

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known γGT activity. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Working temperature	37 °C
Wavelength	405 nm (400-410 nm)
Optical path	1 cm
Reaction	Kinetic (increase)

Allow the reagents to reach working temperature before using.

Bireagent procedure

Pipette into disposable or well clean cuvettes :

	Sample
Reagent A	800 µl
Sample	100 µl
Mix and incubate at 37 °C for 5 minutes, then add:	
Reagent B	200 µl
Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 405 (400-410) nm against water. Read absorbance again 1, 2, 3 minutes thereafter. Calculate ΔA/min.	

Monoreagent procedure

Pipette into disposable or well clean cuvettes :

	Sample
Working reagent	1000 µl
Incubate at 37 °C for 5 minutes, then add:	
Sample	100 µl
Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 405 (400-410) nm against water. Read absorbance again 1, 2, 3 minutes thereafter. Calculate ΔA/min.	

CALCULATION OF RESULTS

Activity (U/l) = ΔA/min x 1111

Note
For values upper than 300 U/l dilute samples 1+9 with saline solution and multiply result by 10.

REFERENCE VALUES

Male	up to 50 U/l
Female	up to 40 U/l

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different enzymatic activities. The obtained results are reported in the following table:

		Within Run		Between Run	
Sample	**Mean (U/l)**	**SD**	**%CV**	**SD**	**%CV**
Serum 1	41.94	0.27	0.6	1.01	2.4
Serum 2	116.15	0.37	0.3	2.14	1.8
Serum 3	188.80	0.90	0.5	2.49	1.3

Linearity

The assay is linear up to 300 U/l.

Sensitivity

Test sensitivity, in terms of limit of detection, is 1 U/l.

Correlation

A correlation study comparing the present method an a commercial one gave the following results:

y = 0.9563x + 2.3692 U/l r = 0.9992

Interferences

Hemoglobin > 500 mg/dl
Bilirubin > 28 mg/dl
Triglycerides > 600 mg/dl

PRECAUTIONS IN USE

Reagent B is harmful.

Refer to Safety Data Sheet.

Reagent A is not considered harmful according to 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

1. SZASZ G. Clin. Chem. 22:2051 (1976)
2. TIETZ Textbook of Clinical Chemistry, Burtis-Ashwood, 2nd Edition (1994)
3. BERGMEYER HU Method of enzymatic analysis (1987)
4. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.
5. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).