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γ-GLUTAMYL TRANSFERASE REAGENT SET (KINETIC) (SOLUBLE SUBSTRATE)

INTENDED USE
γ-Glutamyl Transferase reagent is used for the quantitative determination of γ -GT in human serum.

INTRODUCTION
Gamma-glutamyl transferase (γ-GT) is one of a large group of enzymes known as peptidases. A peptidase is an enzyme that catalyzes the hydrolytic cleavage of peptides to form amino acids or smaller peptides. γ-GT catalyzes the transfer of γ-glutamyl groups from peptides or peptide like compounds to an acceptor peptide molecule. γ-GT was originally termed a "transpeptidase" but the nomenclature was later changed to the more appropriate term "transferase".

Although renal tissue has the highest level of γ-GT, the major source of the enzyme present in serum is of hepatic origin. Elevated levels of γ-GT are found in association with hepatobiliary, pancreatic disorders, alcoholics, myocardial disorders, and diabetics.[1]

Various methods have been employed for the assay of γ-GT activity. Glutathione was used for assessing γ-GT activity in the early time.[2] A synthetic substrate γ-glutamyl-p-nitroanilide (GGPNA) has been used more extensively. Glycylglycine was introduced as an activator and Szasz adapted this activator and the GGPNA to a 30°C kinetic photometric method.[3] Rosalki adapted optimal concentrations of the activator and GGPNA substrate to a 37°C colorimetric endpoint method.[4] Our procedure has been optimized according to Szasz and Rosalki and is performed as either an endpoint or kinetic procedure.

PRINCIPLE
L-γ-glutamyl-p-nitroanilide + glycylglycine
$\xrightarrow{\gamma\text{-GT}}$ p-nitroaniline + y-glutamyl glycylglycine

γ-GT catalyzes the transfer of a γ -glutamyl group from GGPNA. The rate of liberation of p-nitroaniline is directly related to the γ-GT activity in the sample and is quantitated by measuring the increase in absorbance at 405 nm.

REAGENT COMPOSITION
γ-GT reagent (concentrations refer to reconstituted reagent), γ - Glutamyl-p-nitroanilide 4.79 mM, Glycylglycine 100 mM, Tris 180 mM at pH 8.2 ± 0.1.

WARNINGS AND PRECAUTIONS
1. This reagent is for *in vitro* diagnostic use only.
2. *p*-Nitroaniline is toxic and can be absorbed through the skin. *CAUTION:* Avoid ingestion and contact with skin.
3. Do not pipette by mouth.

REAGENT PREPARATION
Reconstitute the reagent with the volume of deionized water stated on the vial. Swirl gently to dissolve.

STORAGE AND STABILITY
Store reagent at 2 - 8°C. Reconstituted reagent is stable for fourteen (14) days at 2 - 8°C (refrigerated).
NOTE: The reagent may have to be re-heated after refrigeration to re-dissolve the substrate.

REAGENT DETERIORATION
The reagent should not be used if:
1. Moisture has entered the vial and caking has occurred.
2. The reconstituted reagent has an absorbance greater than 0.85 at 405 nm when read against water.

SPECIMEN COLLECTION AND STORAGE
Collect whole blood by venipuncture and allow to clot. Centrifuge and remove serum. If not assayed promptly, store samples in refrigerator (2-8°C) or freezer (-20°C). Anticoagulants, particularly fluoride, oxalate and citrate should not be used as they inhibit γ-GT activity. Serum γ -GT is reportedly stable for at least eight (8) hours at 25°C, three (3) days at 2 - 8°C, or one week at -20°C.[5]

INTERFERING SUBSTANCES
1. Anticonvulsant drugs (barbiturates, phenytoin, etc.) may falsely elevate γ-GT values.[6]
2. Young *et al*. give a list of drugs and other substances that interfere with the determination of γ-GT activity.[7]

MATERIALS REQUIRED BUT NOT PROVIDED
1. Pipettes to accurately measure required volumes
2. Timer
3. Test tubes and racks
4. 37°C incubator
5. Spectrophotometer with ability to read at 405 nm and a temperature controlled cuvette

AUTOMATED PROCEDURE
1. Refer to appropriate application manual available.
2. The procedure is run kinetically and is standardized by means of the molar absorptivity of p-nitroaniline taken as 9.90×10^3 at 405 nm under the specified conditions. Results are based on the change in absorbance per minute. All parameters must be known and controlled.

KINETIC PROCEDURE
1. Reconstitute reagent according to instructions.
2. Zero spectrophotometer with distilled water at 405 nm.
3. Set cuvette temperature at 30°C or 37°C.
4. Pipette l.0 ml of working reagent into tubes. Add 0.05 ml (50 µl) of control or patient serum to reagent, mix, and immediately place in the thermo cuvette.
5. Wait thirty seconds and take absorbance measurements at exactly one (1) minute intervals for the next two (2) minutes.
6. Calculate the mean absorbance change per minute (ΔA/min.) and use this figure for value calculation.
7. If above procedure was followed multiply the ΔA/min by the factor 2121.
8. Repeat procedure for each sample.

NOTE: Samples with values above 600 IU/L should be diluted 1:1 with 0.9% saline, re-assay and the final results multiplied by two.

CALCULATIONS

$$\frac{\Delta A/min \times 10^3 \times TV \times 1000}{\in \times SV \times LP} = IU/L$$

ΔA/min.	=	Change in absorbance per minute.
10^3	=	Conversion of millimoles to micromoles.
TV	=	Total reagent volume (ml).
∈	=	Molar absorptivity of p-nitroaniline at 405 nm (9.9×10^3 liters/mole × cm)
SV	=	Sample volume (1.0 ml)
LP	=	Light path (1 cm)

$$\frac{\Delta A/min. \times 10^3 \times 1.05 \times 1000}{9.9 \times 10^3 \times 0.05 \times 1} = \Delta\ A/min \times 2121$$

Example: A sample demonstrated an absorbance change of over a 1-minute interval then

$$0.010 \times 2121 = 21.2\ IU/L.$$

NOTE: If any of the above parameters are changed a new factor must be re-calculated.

DEFINITION OF ENZYME UNIT

One international unit (IU) of γ-GT activity is the amount of enzyme, which transfers 1 µmole of glutamate per minute per liter of sample with the concurrent release of 1 µmole of p-nitroaniline under the specific conditions of the procedure.

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established γ-GT values may be used for quality control. The assigned value of the control material must be confirmed by this methodology. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction or procedural errors.

LIMITATIONS

This procedure is designed to measure γ-GT in serum regardless of its source.

EXPECTED VALUES[8]

Male: 0 - 45 IU/L
Female: 0 - 30 IU/L

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

PERFORMANCE CHARACTERISTICS

1. *Linearity*: 600 IU/L
2. *Sensitivity*: Based on an instrument resolution of 0.001absorbance the present procedure has a sensitivity of 0.02 mg/dl.
3. *Accuracy:* Studies done against reagent of identical methodologies yielded a correlation coefficient of 0.98 and a regression equation y = 0.96 x + 0.39.
4. *Precision:*

   Within Run: Two commercial serum controls were assayed twenty (20) times and the following within run precision was obtained.

	Level I	Level II
Mean	20.2	65.9
S.D.	1.7	2.5
C.V.(%)	8.4	3.8

Run-to-Run: Two commercial serum controls were assayed for five consecutive days (duplicate for each level) and the following run-to-run precision was obtained.

	Level I	Level II
Mean	21.8	65.4
S.D.	1.5	2.8
C.V.(%)	6.8	4.3

REFERENCES

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