GGT Reagent

RAICHEM CE

CLINIQA®

Order No.	Description
R85307	20 × 3 mL
R85240	20 × 6.5 mL
R85241	10 × 20 mL

INTENDED USE

This reagent is intended for the quantitative in vitro measurement of γ-glutamyl transferase (γ-GT) activity in serum or plasma.

INTRODUCTION

Measurements of γ-glutamyl transferase activity are used in the diagnosis and treatment of liver diseases such as alcoholic cirrhosis and primary and secondary liver tumors.

TEST SUMMARY

The first report of an assay for γ-GT employing L-γ-glutamyl-p-nitroanilide as a substrate is the one by Orlowski and Meister (1). This substrate is poorly soluble and for this reason modifications of its formula were sought.

Szasz and Persijn (2) found that the 3-carboxyl derivitive, L-γ-glutamyl-3-carboxy-4-nitroanilide could be substituted for the L-γ-glutamyl-p-nitroanilide, producing a more stable and soluble reagent. The Cliniqa GGT (γ-GT) Reagent utilizes this soluble 3-carboxyl derivative.

Gamma-glutamyl transferase catalyzes the transfer of the glutamyl group from L-γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine with the formation of L-γ-glutamylglycylglycine and 5-amino-2-nitro-benzoate according to the following reaction:

$$\text{L-}\gamma\text{-glutamyl-3-carboxy-4-nitronilide} + \text{glycylclycine} \xrightarrow{\gamma\text{-GT}} \text{L-}\gamma\text{-glutamylglycylglycine} + \text{5-amino-2-nitrobenzoate}$$

The substrate has no color while 5-amino-2-nitrobenzoate absorbs strongly at 405 nm. The amount of 5-amino-2-nitrobenzoate liberated is proportional to γ-GT activity and may be measured kinetically at 405 nm by the increasing intensity of the yellow color formed.

REAGENT COMPOSITION

Reactive ingredients:

L-γ-glutamyl-3-carboxy-4-nitroanilide	3.3 mmol/L
Glycylglycine	110 mmol/L

Non-reactive ingredients:

Buffers, stabilizers and fillers

REAGENT PREPARATION

Dissolve the dry powder in the vial with the volume of distilled or deionized water specified on the vial label. After addition of water, cap vial and mix by gentle inversion until solution is complete.

REAGENT STORAGE AND STABILITY

The unreconstituted dry reagent in the unopened vial is stable until the expiration date on the label when stored in the refrigerator (2–8 °C). The reconstituted reagent is stable for two days at room temperature (22–28 °C) and for 15 days at 2–8 °C.

If the freshly reconstituted reagent has an absorbance at 405 nm, against a water blank, above 0.600 do not use.

PRECAUTIONS

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

SPECIMEN

Serum or EDTA plasma can be used in this assay. Fluoride, oxalate, citrate should not be used, as they inhibit γ-GT activity (3). The enzyme in the sample is stable for at least a week when stored at 2–8 °C (4).

INTERFERENCE

Less than 6 U/L interference (at 37°C) has been demonstrated from 20 mg/dL bilirubin, 250 mg/dL hemoglobin, and 1000 mg/dL Intralipid (representative of lipemia) spiked into normal serum.

It has been found that some antiepileptic drugs (phenytoin, barbiturates) may result in falsely elevated γ-GT values (4). Young (5) has published a comprehensive list of drugs and substances which may interfere with in vitro diagnostic determinations, including γ-glutamyl transferase activity.

MATERIALS REQUIRED BUT NOT PROVIDED

1. A spectrophotometer capable of accurate absorbance measurements at 405 nm.
2. Accurate pipettes to measure distilled or deionized water, reagent and samples.
3. Constant temperature bath. If assay is followed in the cuvette compartment of a spectrophotometer, this should be thermostated.
4. Distilled or deionized water.
5. Matched square cuvettes with 1 cm light path.

MATERIALS PROVIDED

GGT reagent in vials.

TEST PROCEDURE

Wavelength:	405 nm
Temperature:	30 °C

Adjust absorbance to zero with a water blank.

Reagent:	2 mL

Bring to 30 °C temperature.

Add:

Sample:	0.1 mL

Mix by inversion and place in the thermostated cell compartment of the spectrophotometer.

Take readings at 20 second intervals. Determine the ΔA/min. during the linear part of the assay.

QUALITY CONTROL

Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and technique. Commercially available control material with established values for γ-glutamyl transferase activity may be used. Assayed Control Serum, Level 1 (Cat. No. R83082) and Level 2 (Cat. No. R83083) are recommended for this purpose.

CALCULATIONS

$$\Delta\text{A/min.} \times \frac{TV \times 1000}{9.5 \times LP \times SV} = \text{U/L}$$

Where:

TV = Total volume in mL

9.5 = Millimolar extinction of 5-amino-2-nitrobenzoate

LP = Light path (1 if 1 cm cell was used)

SV = Sample volume in mL

1000 converts units/mL to U/L

The factor then is:

$$\frac{2.1 \times 1000}{9.5 \times 0.1} = 2210$$

Multiply the ΔA/min. by 2210 to obtain the activity in U/L in the sample.

Sample Calculations:

If the ΔA/min. was 0.105 the U/L of γ-GT in the sample are:

$$0.105 \times 2210 = 232 \text{ U/L}$$

LIMITATIONS OF THE PROCEDURE

1. The assay can be performed at temperatures different from 30 °C, such as 25 °C or 37 °C. The activity will vary with changes in temperature, but the calculations will remain the same. Expected values will be different.

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2. If the ΔA/min. is greater than 0.235 at 30 °C (activity greater than 500 U/L), dilute the sample with an equal volume of physiological saline (sodium chloride: 150 mmol/L in water) and repeat the assay. Multiply the results by 2.

REAGENT PERFORMANCE

1. Linearity: The assay is linear to 500 U/L.
2. Correlation: Results obtained with this reagent were compared with those obtained using the reagent described by Szasz and Persijn (2). Ninety-three serum samples, ranging in γ-GT activity from 3 U/L to 580 U/L were assayed by the two methods. The correlation coefficient was 0.999 and the regression equation was y = 1x - 0.618.

Results were compared in the same manner with the reagent described by Orlowski and Meister (1) using the same serum samples. The correlation coefficient was 0.999 and the regression equation was y = 1.07x + 1.138.

3. Precision:

Within Run

Mean (U/L)	27.2	78.8	300
SD (U/L)	0.93	0.90	1.69
CV (%)	3.4	1.14	0.56
N	24	24	24

Total

Mean (U/L)	25.3	76.7	299.6
SD (U/L)	0.88	0.96	2.2
CV (%)	3.48	1.25	0.73
N	20	20	20

TEMPERATURE FACTORS

Wahlefeld and Bergmeyer (6) demonstrated that assay procedure described for 30 °C performs equally well at 25 °C and 37 °C. They developed the following table to convert enzyme activities from one temperature to another.

	Factors for conversion to		
Measured	25 °C	30 °C	37 °C
25 °C	1.0	1.35	1.79
30 °C	0.74	1.0	1.32
37 °C	0.56	0.76	1.0

REFERENCE RANGE

The following values were determined. It is reported that γ-GT activity values are some fifty percent higher in adult men than in women or children of both sexes.

Temperature	Female	Male
25 °C	4-18 U/L	6-28 U/L
30 °C	5-24 U/L	8-38 U/L
37 °C	7-32 U/L	11-50 U/L

REFERENCES

1. Orlowski, M., Meister, A., *Biochem. Bioph Acta* 73, 679, 1963.
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3. Whitfield, J.B., Moss, D.W., Neale, G., Orme, M. and Breckenridge, A., *Brit. Med. J.* 1, 316, 1973.
4. Szasz, G., *Clin Chem* 15:124, 1969.
5. Young, D.S., *Effects of Drugs on Clinical Laboratory Testing,* 3rd Edition, 3.183-3.185, AACC Press, Washington, DC, 1990.
6. Wahlefeld, A.W. and Bergmeyer, H.U., in *Methods* of *Enzymatic Analysis,* Third Edition, Vol. III, p. 352, H.U. Bergmeyer, Editor, Verlag Chemie, Deerfield Beach, FL, 1983.

IVD **For in vitro diagnostic use**

See package insert for proper use

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RE-ORDER INFORMATION
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Catalog No.	Catalog No.	Catalog No.
REF R85240	REF R85241	REF R85307

Made in the USA

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