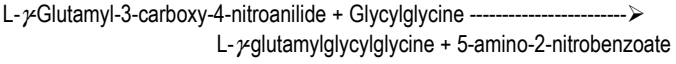
POINTE SCIENTIFIC, INC.
5449 Research Drive • Canton MI 48188 • USA

Liquid γ-GT (γ-Glutamyl Transferase) Reagent Set

Intended Use
For the quantitative kinetic determination of gamma glutamyl transferase (GGT) activity in serum. For *in vitro* diagnostic use only.

Clinical Significance
γ-GT measurements are used in the diagnosis and treatment of liver diseases such as alcoholic cirrhosis, and primary and secondary tumors. Elevated γ-GT levels appear earlier and are more pronounced than those of other liver enzymes in cases of obstructive jaundice and metastatic neoplasms.[1]

Method History
Methods for determining γ-GT are based on the use of glutamyl derivatives of aromatic amines as substrate material.[2] Orlowski and Meister introduced γ-glutamyl-ρ-nitroanilide as a substrate in 1963[3] with Kulhanek and Dimov (1966) adding glycylglycine and significantly increasing the speed of the reaction.[4] In 1969, Szasz published a kinetic procedure for γ-GT [5] on whose principle the present procedure is based. Szasz and Persijn[6] later reported that the 3-carboxyl derivitive, L-γ-glutamyl-3-carboxy-4-nitroanalide (GLUPA-C) could be substituted for the L-γ-glutamyl-ρ-nitoanilide, producing a more stable reagent. The Pointe Scientific Liquid γ-GT reagent uses this soluble 3-carboxyl derivative.

Principle


γ-GT in the sample catalyzes the transfer of the glutamyl group from GLUPA-C to glycylglycine according to the above reaction. The amount of 5-amino-2-nitrobenzoate formed is proportional to γ-GT activity and may be measured kinetically.

Reagent Composition
In addition to a stabilizer, the combined R1 and R2 reagent contains:

Tris buffer (pH 8.1 ±0.1)	89 mmol/L
Glycylglycine	126 mmol/L
GLUPA-C	3.2 mmol/L
Sodium Azide	0.095%

Reagent Preparation
Reagents are supplied as ready to use liquids.

Reagent Storage and Stability
Store reagents at 2-8°C. The reagents are stable until the expiration date if stored as directed. **NOTE:** The R2 reagent is temperature sensitive and can be affected by prolonged exposure to room temperature.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Do not pipette by mouth. Avoid ingestion and contact with skin as toxicity has not been established.
3. Reagents in this kit contain sodium azide as a preservative. Sodium azide may form explosive compounds in metal drainlines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CSC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

Specimen Collection and Storage
1. Use serum only. γ-GT activity is inhibited by most anticoagulants.
2. It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.
3. Serum γ-GT is reported stable in serum for up to seven days when stored at 20-25°C, up to one month when stored at 4°C, and up to one year at -20°C and protected from evaporation.[7]
4. All specimens and controls should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Ed., 1988, HHS Publication No. (CDC) 88-8395.

Interferences
1. Most anticoagulants used in blood collection tubes inhibit γ-GT activity.[8]
2. Anti-epileptic drugs (phenytoin and barbituates) may falsely elevate γ-GT levels.[9,10]
3. Bilirubin to the level of 6.8 mg/dl has been found to exhibit negligible interference (< 10%) in this assay.
4. Hemoglobin to 375 mg/dl has been found to show minimal depression (<10%) of recovered γ-GT activities.
5. For a comprehensive list of drug interferences, see Young et al.[11]

Materials Provided
γ-GT reagents (R1 and R2).

Materials Required but not Provided
1. Beckman Coulter AU™ analyzer.
2. Chemistry Controls
3. Instrument application and Operation manual.

Procedure (Beckman Coulter AU™400 application)

SPECIFIC TEST PARAMETERS

TEST NUMBER: # TEST NAME: **GGT** ∇ TYPE: **Serum** ∇ OPERATIONAL: **Yes** ∇
SAMPLE VOL.: **6** DIL. VOL.: **0** PRE-DILUTION RATE: **1**
REAGENTS: R1 VOLUME: **150** DIL. VOL.: **0** MIN. OD MAX. OD
R2 VOLUME: **60** DIL. VOL.: **0** L **-2.000** H **2.500**
REAGENT OD LIMIT:
WAVELENGTH: PRI. **410** ∇ SEC. **480**∇ FIRST L: **0.000** FIRST H: **2.500**
METHOD: **RATE** ∇ LAST L: **0.000** LAST H: **2.500**
REACTION SLOPE: **+** ∇ DYNAMIC RANGE:
MEASURING POINT 1: FIRST: **20** LAST: **27** L: **#** H: **#**
MEASURING POINT 2: FIRST: LAST: CORRELATION FACTOR:
LINEARITY: **25** % A: **1.000** B: **0.000**
NO LAG TIME: **NO** ∇ ON BOARD STABILITY PERIOD: **#**

SPECIFIC TEST PARAMETERS

VALUE FLAG: **#** ∇ LEVEL L: **#** LEVEL H: **#**

NORMAL RANGES:	SEX	AGE L YEAR	AGE L MONTH	AGE H YEAR	AGE H MONTH	L	H
○ 1.	# ∇	#	#	#	#	#	#
○ 2.	# ∇	#	#	#	#	#	#
○ 3.	# ∇	#	#	#	#	#	#
○ 4.	# ∇	#	#	#	#	#	#
○ 5.	# ∇	#	#	#	#	#	#
○ 6.	# ∇	#	#	#	#	#	#
7. NONE SELECTED						#	#
8. OUT OF RANGE	L H					#	#

PANIC VALUE: # # UNIT: **U/L** DECIMAL: PLACES: **0**

Liquid γ-GT (γ-Glutamyl Transferase) Reagent Set

CALIBRATION SPECIFIC PARAMETERS

CAL TYPE: **MB** ∇ FORMULA: **Y=AX+B** ∇ COUNTS: **2** PROCESS: **CONC.** ∇

	CAL. NO.	OD	CONC.	FAC/OD-L	FAC/OD-H
POINT 1.	#		#	**-9999999**	**9999999**
POINT 2.					
POINT 3.					
POINT 4.					
POINT 5.					
POINT 6.					
POINT 7.					

1-POINT CAL. POINT: ○ WITH CONC-0

MB TYPE FACTOR: **4712** CALIBRATION STABILITY PERIOD: **#**

#: User-Defined

The above reagent parameters are intended to serve as a guide for use with Pointe Scientific, Inc. reagent. The parameters are based on data generated by Pointe Scientific, Inc. Please note: These parameters should be used in conjunction with your laboratory Quality Control Program for validation.

NOTE: For other instrument specific applications please contact Pointe Scientific, Inc. Technical Service Department at 1-800-445-9853

Limitations

Samples that exceed the linearity limit (800 U/L) should be diluted with an equal volume of saline and re-assayed and the final results multiplied by two.

Calibration

The procedure is calibrated by means of the millimolar absorptivity of 5-amino-2-nitrobenzoate which is 9.5 under the specified conditions. Results are based on the change in absorbance per minute. All parameters must be known and controlled.

Calculations

γ-GT activity is expressed as units/liter. At 37°C, one Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under defined conditions.

$$\frac{\Delta \text{ Abs/min} \times TV \times 1000}{MMA \times SV \times LP} = \text{U/L } \gamma\text{-GT in sample}$$

Δ Abs/min……Change in absorbance per minute
TV……Total assay volume (1.100ml)
1000……Conversion of ml to L
MMA……millimolar absorptivity of 5-amino-2-nitrobenzoate(9.5)
SV……Sample volume (0.100ml)
LP……Light path (1cm)

$$\frac{\Delta \text{ A/min} \times 1.100 \times 1000}{9.5 \times 0.100 \times 1.0} = \Delta \text{ A/min}$$

Then: Δ A/min x 1158 = U/L of unknown

Example: If Δ A/min = .06, then .06 x 1158 = 69 U/L

Note: If any of the above parameters are changed, a new factor must be recalculated.

Quality Control

The validity of the reaction should be monitored by the use of control sera with known normal and abnormal γ-GT values. These controls should be run at least with every working shift in which γ-GT assays are performed. It is recommended that each laboratory establish its own frequency of control determination.

Expected Values [12]

Male: 8-37 U/L at 30°C, 9-54 U/L at 37°C
Female: 6-24 U/L at 30°C, 8-35 U/L at 37°C
Due to a wide range of conditions (dietary, geographical, age, etc.) known to affect normal ranges, it is strongly recommended that each laboratory determine its own reference range.

Performance

1. Linearity: 800 IU/L. Samples that exceed 800 U/L should be diluted with an equal volume of saline and re-assayed. Multiply the result by two.
2. Comparison: A comparison study performed between the Beckman Coulter AU™400 and Roche Hitachi 717 using this method resulted in a correlation coefficient of r = 0.996 and a regression equation of y=0.996x +2.66. (n = 28, range 6 – 97 IU/L))
3. Precision:
   Within - day precision study was performed using three levels of material.
   Between - day precision study was performed using two levels of control material assayed over a 20 day period with 2 runs per day and 2 replicates per run.

Within Day (N=20)			Day to Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
39	0.5	1.3	38	2.6	6.8
322	2.9	0.9	127	0.5	0.4
614	6.6	1.1			

   Precision and Linearity studies were performed following modifications of CLSI Protocols EP-5 and EP6[13] using a Beckman Coulter AU™400 analyzer.
4. Sensitivity: The sensitivity for the liquid γ-GT reagent was investigated by reading the change in absorbance at 410 / 480 nm for a saline sample, and serum samples with known concentrations. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used, the liquid γ-GT reagent showed little or no drift on a zero sample. Under the reaction conditions described, 1 U/L gives an absorbance movement of 0.0002.

References

1. Tietz, N.W., editor, Fundamentals of Clinical Chemistry, 3rd Ed., W.B. Saunders Co., p. 391 (1987).
2. Demetriou, J.A., Drewes, P.A., Gin, J.B., Clinical Chemistry: Principles and Technics, 2nd Ed., Hagerstown (MD), Harper Row, pp. 872-873 (1974).
3. Orlowski, M., Meister, A., Biochem, Biophys. Acta 73:679 (1963).
4. Kulhanek, V., Dimov, D.M., Clin. Chem. Acta 14:619 (1966).
5. Szasz, G., Clin. Chem. 15:124 (1969).
6. Szasz, G., Persijn, J.P., et al, A Klin. Chem. Klin. Biochem. 12:228 (1974).
7. Zern, M., and Discombe, G., Lancet 2:748 (1971).
8. Wolf, P.L., et al, Practical Clinical Enzymology and Biochemical Profiling, New York, Wiley-Interscience p. 37 (1973).
9. Rosalki, S.B., et al, Lancet 2:376 (1971).
10. Whitfield, J.B., et al, Gut 13:702(1972).
11. Young, D.S., et al, Clin. Chem. 21:1D (1975).
12. Kaplan, L.A., Pesce, A.J. Clinical Chemistry, 2nd Ed., St. Louis, C.V. Mosby Company, (1992).
13. CLSI protocols EP5-A2, Vol. 24 No. 25, 2nd Ed, (2004) and CLSI EP6-A, Vol. 23 No. 16 (2003)

Manufactured by Pointe Scientific, Inc.
5449 Research Drive, Canton, MI 48188

European Authorized Representative:
Obelis s.a.
Boulevard Général Wahis 53
1030 Brussels, BELGIUM
Tel: (32)2.732.59.54 Fax:(32)2.732.60.03 email: mail@obelis.net

CE

Rev. 12/12 P803-OG959-01