Gamma GT Reagent - IFCC Standardized

for Beckman Coulter™ SYNCHRON‡ and UniCel‡ Systems‡

REF A46660 (2 x 150 tests)

INTENDED USE

This reagent is intended for the in vitro quantitative determination of Gamma Glutamyl Transferase (GGT) [(γ-Glutamyl) - Peptide: Amino Acid γ-Glutamyltransferase, EC2.3.2.2], in human serum or plasma on Beckman Coulter Synchron CX/LX and UniCel DxC Systems. The calculation factor applied to this procedure will result in assay values which are comparable with the method recommended by the IFCC.[1]

CLINICAL SIGNIFICANCE

Although GGT is present in a variety of tissues, the serum enzyme appears to be primarily from the hepato-biliary system. Consequently, GGT is elevated in all forms of liver disease or damage. It is clinically useful in detecting obstructive jaundice, cholangitis, and cholecystitis. Elevated levels are also observed with drug use (alcohol, sedatives, anticonvulsants and tranquilizers).[2]

METHODOLOGY

The first commercially available kinetic methods for the determination of GGT were based on the work of Szasz[3], Rosalki and Tarlow[4]. These methods utilised γ-glutamyl-p-nitroanilide (Glu-4-NA) as the substrate, however the poor solubility and stability of Glu-4-NA was a major limitation.

In order to improve the method Persijn[5] investigated further with Glu-4-NA derivatives and found that γ-glutamyl-3-carboxy-4-nitroanilide (Glucana) was superior to the Glu-4-NA with respect to both solubility and stability. The Glucana substrate now forms the basis of the IFCC and ECCLS recommended procedures.

The GGT- IFCC Standardized method utilises Glucana in the following reaction, which is initiated by the addition of sample. GGT present in the sample catalyses the transfer of the glutamyl group from the substrate to glycylglycine forming glutamylglycylglycine and 5-amino-2-nitrobenzoate.

L-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine

↓ *GGT*

L-γ-glutamylglycylglycine + 5-amino-2-nitrobenzoate

The rate of formation of 5-amino-2-nitrobenzoate is proportional to the activity of GGT present in the sample and can be measured kinetically at 410 nm.

REAGENT COMPOSITION

Active Ingredients	Concentration
Reagent A(Compartment A)	
Glycylglycine	130 mmol/L
NaCl	65 mmol/L
Also contains non-reactive fillers and stabilizers	
Preservative	
Reagent B (Compartment B)	
L-γ-glutamyl-3-carboxy-4-nitroanilide	20 mmol/L
Preservative	

pH 8.15 ± 0.1 at 20°C.

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt thoroughly wash affected areas with water. Reagent contains Sodium Azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the GGT Reagent Material Safety Data Sheet.

REAGENT PREPARATION

Reagent is supplied ready to use. Transfer the contents of Reagent A and Reagent B into appropriate compartments of the User Defined Reagent (UDR) cartridge included in the kit as shown in the table below. Use care to avoid contamination.

GGT Kit	Compartment A	Compartment B
Reagent A	39 mL	-
Reagent B	-	9.4 mL

STABILITY AND STORAGE

The unopened reagents are stable until the expiration date when stored at 2-8°C. When stored on Synchron LX/CX and UniCel DxC Systems, the reagent is stable for 14 days.

Indications of Reagent Deterioration:

- Turbidity
- Failure to recover control values within the assigned range
- "BL ABS HI" flag.

SPECIMEN COLLECTION AND HANDLING

Collection: It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.[6]
Serum: Use non-haemolysed serum.
Plasma: Li-heparin or Na-heparin.
Storage: GGT Reagent is stable for 7 days when stored at 2-8°C.[3]

MATERIALS PROVIDED

- Thermo Scientific GGT Reagent for Beckman Coulter Synchron LX/CX and Unicel DxC Systems.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- Beckman Coulter Synchron LX/CX and Unicel DxC chemistry analyzer.
- Beckman Coulter sample cups.
- Assayed Normal and Abnormal Controls.

TESTING PROCEDURES

Load the reagent onto the system as directed in the Operations Manual. Program samples and controls for analysis as directed in the Operations Manual.
For Synchron LX/CX and Unicel DxC System parameters refer to the System Parameters section of this package insert

CALIBRATION

Calibration is not required. The Synchron LX / CX and Unicel DxC Systems calculates U/L of activity by multiplying the measured rate of reaction by the programmed Calculation Factor (Refer to the System Parameters Section of this package insert). The calculation factor has been derived to provide traceability to the IFCC GGT reference measurement procedure.[1]

CALCULATIONS

Results are calculated automatically by the Synchron LX / CX and Unicel DxC Systems.
Unit conversion: U/L x 16.67 x 10^{-3} = µkat/L.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:

- At least once per day or as established by the laboratory.
- When a new reagent cartridge is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate that the assay may be out of control. The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Beckman Coulter Technical Services or your local distributor.

LIMITATIONS

1. Analytical specificity studies to determine the level of interference from various sample components were carried out on the CX and LX /DxC analyzers. No interference was observed below the following interferent concentration limits (pass criterion, initial control value ± 10%):

	CX	LX / DxC
Haemoglobin	500 mg/dL	500 mg/dL
Lipaemia	1000 mg/dL	1000 mg/dL
Unconjugated Bilirubin	60 mg/dL	60 mg/dL
Conjugated Bilirubin	60 mg/dL	60 mg/dL

2. Young DS[7] has published a comprehensive list of drugs and substances which may interfere with this assay.

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EXPECTED VALUES[1]

Male At 37°C < 55 U/L (0.917 µkat/L)
Female At 37°C < 38 U/L (0.633 µkat/L)

The quoted values should serve as a guide only. It is recommended that each laboratory verify this range or derive a reference interval for the population that it serves.[8]

PERFORMANCE DATA

The following data was obtained using Thermo Scientific GGT Reagent on the Beckman Coulter Synchron LX / CX and Unicel DxC Systems according to established procedures.

IMPRECISION

Precision was evaluated using the NCCLS (CLSI) EP5-A2 guideline.[9] Studies to represent typical performance on a well maintained analyser were carried out at the same site over a period of 20 days (40 runs) using three levels of commercially available quality control serum. Two runs per day were carried out by the same operator on the same lots of reagent, on single CX and LX/DxC analyzers.

CX Imprecision		Level 1		Level 2		Level 3	
		U/L	µkat/L	U/L	µkat/L	U/L	µkat/L
n		80		80		80	
Mean		47	0.790	220	3.66	421	7.01
Within Run	SD	3.8	0.063	4.9	0.082	4.8	0.080
	CV %	8		2.2		1.1	
Total	SD	4.9	0.082	7.1	0.118	11.4	0.190
	CV %	10.3		3.2		2.7	

LX / DxC Imprecision		Level 1		Level 2		Level 3	
		U/L	µkat/L	U/L	µkat/L	U/L	µkat/L
n		80		80		80	
Mean		50	0.837	225	3.75	436	7.26
Within Run	SD	3.4	0.057	3.5	0.058	5.0	0.083
	CV %	6.8		1.5		1.1	
Total	SD	4.0	0.067	5.2	0.087	7.0	0.117
	CV %	8		2.3		1.6	

METHOD COMPARISON

Comparison studies were carried out using the NCCLS (CLSI) EP9-A2 guideline.[10] A commercially available GGT reagent traceable to the IFCC method was used as the reference method (X), using recommended applications on a Roche Hitachi 911® analyzer. The test method (Y) was run with recommended applications on the Beckman Coulter Synchron LX / CX and UniCel DxC analyzers. Serum / plasma samples were assayed in parallel by both the test and reference methods and the results compared by Deming regression. The following statistics were obtained:

Serum/ Plasma	CX		LX / DxC	
	U/L	µkat/L	U/L	µkat/L
n	92		104	
Range	8 - 949	0.133 - 15.8	4 -985	0.067 - 16.4
X-mean	116	1.93	119	1.98
Y-mean	115	1.92	126	2.10
Slope	0.971		1.00	
Intercept	2.4	0.040	6.6	0.110
r	0.999		0.9994	

MEASURING RANGE

When run as recommended the measuring range of the assay is as follows:

CX: 7 - 1200 U/L (0.117 - 20.0 µkat/L)
LX/DxC: 4 - 1200 U/L (0.067 - 20.0 µkat/L)

LIMIT OF DETECTION

The limit of detection represents the lowest measurable analyte level that can be significantly distinguished from zero. It is calculated as the value lying two standard deviations above the mean estimate for an appropriate zero (blank) sample.
When run as recommended the limit of detection is:

CX: 7 U/L (0.117 µkat/L)
LX/DxC: 4 U/L (0.067 µkat/L)

ADDITIONAL INFORMATION

Since Beckman Coulter does not manufacture the reagent or perform quality control or other tests on individual lots, Beckman Coulter cannot be responsible for the quality of the data obtained which is caused by performance of the reagent, any variation between lots of reagent, or protocol changes by the manufacturer.

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REFERENCES

1. Schumann, G et al. "IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37°C.Part 6. Reference Procedure for the Measurement of Catalytic Concentration of γ–Glutamyltransferase. Clin Chem Lab Med 2002;40:734-8.
2. Kachmar JF, Moss DV. "Enzymes" in Fundamentals of Clinical Chemistry. Tietz NW (Ed) WB Saunders Co. Philadelphia 1976; page 621-3.
3. Szasz G. Clin Chem 1969; 15: 124-36.
4. Rosalki SB, Tarlow D. Clin Chem 1974; 20: 1121-4.
5. Persijn JP and van der Slik W. J. Clin Chem Clin Biochem. 1976; 14: 421-7.
6. National Committee for Clinical Laboratory Standards.'Procedures for the Handling and Processing of Blood Specimens. Approved Guidline, NCCLS publication C28-A, Villanova, PA.1990.
7. Young DS. Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990; 3: 183-5.
8. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.
9. Tholen, D. W. et. al. 'EP5-A2. Evaluation of precision performance of quantitative measurement methods; Approved guideline – second edition. National Committee for Clinical Laboratory Standards. 2004; Volume 24: Number 25.
10. Krouwer, J. S. et al. 'EP9-A2. Method comparison and bias estimation using patient samples; Approved guideline – second edition. National Committee for Clinical Laboratory Standards. 2002; Volume 22: Number 19.

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SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		
	Consult instructions for use		Manufactured by
REAG A	Reagent A	REAG B	Reagent B
			NotReUse

Chemistry Parameters for Synchron CX series Systems

Number [*] Chem [GGTX]

Chemistry Parameters		Page 1 of 3
Reaction Type	Rate 1	
Units	U/L	
Precision	XXXX	
Reaction Direction	[Positive]	
Math Model	[Linear]	
Primary Wavelength	[410]	
Secondary Wavelength	[650]	
Calculation Factor	[11009]	
No. of Calibrators	[0]	
Setpoints	1 [ ]	4 [ ]
	2 [ ]	5 [ ]
	3 [ ]	6 [ ]
Cal Time Limit	[0] hours	
Cal Save	[√]	

Processing Parameters		Page 2 of 3
First Inject	Component	[A]
	Dispense Volume	[210] µL
Second Inject	Component	[B]
	Dispense Volume	[53] µL
	Inject Time	[0] sec.
Third Inject	Component	[None]
	Dispense Volume	[0]
	Inject Time	[N/A]
Sample Volume	[7] µL	
ORDAC Volume	[ ] µL	

<F2 Dilute>		
Neat Sample Volume	[ ]	
Diluent§ Volume	[ ]	
Dilution Factor	15.0 (automatic)	
Sample Diluent	[√] DIL 1 Cartridge	
Blank	Start Read	[80] sec
	End Read	[140] sec
Initial (DxC only)	Start Read	[ ] sec
	End Read	[ ] sec
Reaction 1	Start Read	[60] sec
	End Read	[294] sec
Reaction 2	Start Read	[ ] sec
	End Read	[ ] sec

Error Detection Limits		Page 3 of 3
Blank	ABS Low/High Limits	[-0.1]/[0.65]
	Rate Low/High Limits	[ ]/[ ]
	Mean Deviation	[ ]
Reaction 1	ABS Low/High Limits	[-0.1]/[1.5]
	Rate Low/High Limits	[ ]/[ ]
	Mean Deviation	[ ]
Reaction 2	ABS Low/High Limits	[ ]/[ ]
	Rate Low/High Limits	[ ]/[ ]
	Mean Deviation	[ ]
Substrate Depletion		
	Initial Rate	[99.999]
	Delta ABS	[1.5]
Multipoint Span		
	1-2	[0.000] [ ]
		[ ] [ ]
		[ ] [ ]
Usable Result Range		
	Low Limit	[7]
	High Limit	[1200]
ORDAC		
	Low Limit	[ ]
	High Limit	[ ]

Chemistry Parameters for Synchron LX series and UniCel DXC series Systems

Number [*] Chem [GGTX]

Chemistry Parameters		Page 1 of 3
Reaction Type	Rate 1	
Units	U/L	
Precision	XXXX	
Reaction Direction	[Positive]	
Math Model	[Linear]	
Primary Wavelength	[410]	
Secondary Wavelength	[650]	
Calculation Factor	[11009]	
No. of Calibrators	[0]	
Setpoints	1 [ ]	4 [ ]
	2 [ ]	5 [ ]
	3 [ ]	6 [ ]
Cal Time Limit	[0] hours	
Cal Save	[√]	

Processing Parameters		Page 2 of 3
First Inject	Component	[A]
	Dispense Volume	[210] µL
Second Inject	Component	[B]
	Dispense Volume	[53] µL
	Inject Time	[-180] sec.
Third Inject	Component	[None]
	Dispense Volume	[0]
	Inject Time	[N/A]
Sample Volume	[7] µL	
ORDAC Volume	[ ] µL	

<F2 Dilute>		
Neat Sample Volume	[ ]	
Diluent§ Volume	[ ]	
Dilution Factor		
Sample Diluent	[√] DIL 1 Cartridge	
Blank	Start Read	[-100] sec
	End Read	[-40] sec
Initial (DxC only)	Start Read	[ ] sec
	End Read	[ ] sec
Reaction 1	Start Read	[60] sec
	End Read	[294] sec
Reaction 2	Start Read	[ ] sec
	End Read	[ ] sec

Error Detection Limits		Page 3 of 3
Blank	ABS Low/High Limits	[-0.1]/[0.65]
	Rate Low/High Limits	[-1.500]/[2.200]
	Mean Deviation	[2.200]
Reaction 1	ABS Low/High Limits	[-0.1]/[2.2]
	Rate Low/High Limits	[-1.500]/[2.200]
	Mean Deviation	[2.200]
Reaction 2	ABS Low/High Limits	[ ]/[ ]
	Rate Low/High Limits	[ ]/[ ]
	Mean Deviation	[ ]
Substrate Depletion		
	Initial Rate	[99.999]
	Delta ABS	[2.200]
Multipoint Span		
	1-2	[0.000] [ ]
		[ ] [ ]
		[ ] [ ]
Usable Result Range		
	Low Limit	[4]
	High Limit	[1200]
ORDAC		
	Low Limit	[ ]
	High Limit	[ ]