ROBONIK®

CE

prietest™...Clinical Chemistry Reagents

ALT/SGPT TEST KIT

INTENDED USE :
- Quantitative in vitro determination of Activity of GPT/ALT in serum or plasma on photometric systems.
- In vitro diagnostic test kit, for laboratory and professional use.
- This manual contains instructions for operation by qualified personnel only.

ORDERING INFORMATION	**Pack Size**	**Cat No.**
	1 X 25 ml	GPT 01 25
	2 X 50 ml	GPT 02 50
	4 X 50 ml	GPT 04 50
	2 X 500 ml	GPT 02 500

CLINICAL SIGNIFICANCE : Glutamate Pyruvate Transaminase (GPT) also known as Alanine aminotransferase (ALT) is a transaminase. GPT catalyses the transfer of the amino group of L-alanine to α-ketoglutarate to give L-glutamate. The highest levels are found in the liver and the kidneys, and in smaller amounts in heart and skeletal muscle.
GPT concentration is increased when hepatic cells are damaged (liver cell necrosis or injury of any cause). Indeed, viral and toxic hepatitis induce a marked elevation of GPT activity in serum. Intake of alcohol, delirium tremens, and administration of various drug induce slight or moderate elevation of GPT. GPT concentration in serum is also slightly increased in various conditions such as : muscular dystrophy, hemolytic disease, myocardial infarction... GPT is more liver specific than GOT (Aspartate aminotransferase). Measurement of both GOT and GPT has some value in distinguishing hepatitis from other parenchymal lesions. GPT serum level can decrease in case of vitamin B6 deficiency.

METHOD : IFCC method without pyridoxal phosphate , Kinetic, UV.

PRINCIPLE : Kinetic determination of the GPT activity :

$$\text{L - Alanine} + \alpha\text{ - Ketoglutarate} \xrightarrow{\text{GPT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + \text{NAD}^+$$

REAGENTS :
COMPONENTS AND CONCENTRATIONS:

R1 :	Tris Buffer	:	100 mmol/l
	L - Alanine	:	500 mmol/l
	Lactate Dehydrogenase	:	> 1200 U/l
R2 :	α - Ketoglutarate	:	150 mmol/l
	NADH	:	0.18 mmol/l
	Preservative & Stabilizer		

STORAGE INSTRUCTIONS AND REAGENT STABILITY : The reagents are stable up to the end of the indicated date of expiry on the vial label, if stored at 2 to 8°C, protected from light and contamination is avoided. Do not freeze the reagent ! Discard the Reagent if found turbid or in case the absorbance of Working Reagent is less than 1.0 AU at 340 nm against distilled water.

WARNINGS AND PRECAUTIONS : Take the necessary precautions for the use of laboratory reagents.

WASTE MANAGEMENT : For disposal of these biomedical waste refer local biosafety regulations.

REAGENT PREPARATION :
1) Two Reagent procedure : The reagents are ready-to-use.
2) One Reagent Procedure : Mix four volumes of reagent R1 with one volume of reagent R2. Stability of working reagent solution : Four Weeks at 2 to 8°C

MATERIAL REQUIRED BUT NOT PROVIDED : NaCl solution 9 g/l, General laboratory equipment, Analyser / Photometer, pipettes etc.

SPECIMEN :
Serum free from hemolysis, Heparinized or EDTA plasma.
Stability in serum / plasma
Sera are stable 24 hours at 20 to 25 °C , 28 days at 4°C.
Discard contaminated specimens.

ASSAY PROCEDURE 1: Two Reagent procedure
Application sheets for automated systems are available on request.

Wavelength	:	Hg 340 nm
Optical path	:	1 cm
Temperature	:	37°C
Mode	:	Kinetic

Bring all the contents of the kit to Room Temperature prior to use.
Read rate of change of absorbance of sample against distilled water or air.
Label the test tube as sample, control and pipette into respective test tube the reagent, sample, control sample as per the table given below :

	Sample / Control
Reagent R1	800 µl
Reagent R2	200 µl

Mix and Incubate at 37°C for 2 minutes then add

Sample / Control	100 µl

Mix and after a 60 seconds incubation at 37°C measure the change of absorbance per minute (ΔA/minute) during 180 seconds.

ASSAY PROCEDURE 2: One Reagent procedure
Label the test tube as sample, control and pipette into respective test tube the reagent, sample, control sample as per the table given below :
Prewarm working reagent at 37°C for two minutes prior to addition of sample.

	Sample / Control
Working Reagent	1000 µl
Sample / Control	100 µl

Mix and after a 60 seconds incubation at 37°C measure the change of absorbance per minute (ΔA/minute) during 180 seconds.

CALCULATION : At 340 nm with one reagent procedure and two reagent procedure for 1 cm path light cuvette
Activity of Sample (U/L) = (Δ A/Min) X 1746

TEMPERATURE CONVERSION FACTORS :
To correct result to other temperatures multiply by factor shown in table

Assay Temperature	**Conversion Factor to**		
	25°C	30°C	37°C
25°C	1.00	1.37	2.08
30°C	0.73	1.00	1.54
37°C	0.48	0.65	1.00

CALIBRATION : For the calibration of automated photometric systems use of the commercially available calibrator is recommended.

QUALITY CONTROL : To ensure adequate quality, use of the commercially available control sera is recommended.

PERFORMANCE CHARACTERISTICS :
MEASURING RANGE : The test has been developed to determine GPT/ALT Activity within a measuring range from 5 to 400 U/L. When values exceed higher limit of the range, such samples should be diluted 1 + 1 with NaCl solution (9 g/l) and the result multiplied by 2.

SPECIFICITY/ INTERFERENCE : No interference was observed by Ascorbic Acid up to 30 mg/dl(1703.4 µmol/L), Bilirubin up to 15 mg/dl(256.5 µmol/L), Glucose up to 500 mg/dl(27.77 mmol/L), and lipemia up to 600 mg/dl(6.78 mmol/L), Triglycerides. A list of drugs and other interfering substances with GPT/ALT determination has been reported by Young et al.

SENSITIVITY/ LIMIT OF DETECTION : The lower limit of detection is 5 U/L.

PRECISION :

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	48.7	0.3	0.61
Sample 2	105	1.0	0.95
Sample 3	108	1.05	0.97

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	19.3	0.15	0.77
Sample 2	36.2	0.3	0.83
Sample 3	135	1.0	0.74

METHOD COMPARISON :
A comparison between Robonik Prietest GPT/ALT (y) and a commercially available test (x) using 20 samples gave following results:
Linear Regression : y = 1.1429 x + 0.812 U/L **Correlation Coefficient** : r = 0.9909

REFERENCE RANGE :
Men : 0 to 40 U/L **Women** : 0 to 32 U/L
It is recommended that each laboratory should assign its own reference range.

LITERATURE :

1. Johnson AM, Rohlfs EM, Silverman LM. Proteins. In: Burtis CA, Ashwood ER. Editors Tietz textbook of clinical chemistry. 3rd ed. Philadelphia : W.B. Saunders Company; 1999. p. 477-540.
2. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt : TH-Books Verlagsgesellschaft; 1998. p. 652-6.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed. AACC Press, 1995

INSTRUMENT APPLICATION *prietest* INSTRUMENTS	
Name : GPT,	Mod : KIN
Pri.: 340,	Sec.: 0
Temp: 37C,	KF : 1.000
Vol : 500ul,	Unit : U/L
Lag : 60,	Read : 180
Blk : N, QC : Y,	Norm : Y
Std : N,	Factor : 1746
Normal HI = 40	
Normal LO = 0	
QCNH : *	
QCNL : *	
QCABH = *	
QCABL = *	
Init. OD : = 1.0 L	
Max Delta/Min : = 0.229	
Rgnt. Linearity : 400	
NOTE :	
* Indicates user definable parameter.	
NA Implies Not Applicable	

PARAMETERS FOR INSTRUMENT SETTING	
TEST NAME	GPT
Reaction	Kinetic
Reaction Slope	Decreasing
Wavelength 1	340 nm
Temperature	37°C
Zero Setting	Distilled Water
Factor	1746
Units	U/L
Sample Volume	100 µl
Reagent Volume	1000 µl
Lag Time	60 Seconds
Read Time	180 Seconds
Reference Range	0 to 40
Reagent Linearity	400
Max Delta/Min	0.229
Initial OD	> 1.0

ROBONIK®

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IVD For in vitro diagnostic use | Store at | Consult Instructions for use | REF Catalogue Number | Exp. Date | LOT Lot No. | Manufacturer's Address | Date of Manufacture

Ver.: PGPT/ALT/0618/01