

# **SGPT KIT**

Method: Modified IFCC Method

### PRINCIPLE:

The enzymatic reaction sequence employed in the assay of SGPT is as follows

ALT

L - Alanine 2+ oxoglutarate Pyruvate + L - Glutamate

Pyruvate + NADH + H<sup>+</sup> LDH D-Lactate + NAD<sup>+</sup>

### **REAGENTS:**

Reagent R1 : Ready - To - Use
Reagent R2 : Ready - To - Use

## **REAGENT PREPARATION:**

Mix 4 parts of R1 with 1 part of R2. The combined reagent is stable for 5 days at 15<sup>0</sup> to 25<sup>0</sup> C; 2 weeks at 2-8<sup>0</sup> C. Protect mixed reagent from light.

## STORAGE & STABILITY:

Store at 2-8°C and protect from light.

Avoid Contamination of Ready - To - Use Reagents. Always use fresh pipette tips. Keep always the caps tightly closed

The above reagents R1 & R2 are Ready – To – Use and are stable until expiry dates mentioned on the label.

Note- Preincubate the reagents for 5 min at 370 C

## **COLLECTION AND HANDLING OF SPECIMEN:**

- 1. Avoid hemolysis as red cells contain ALT-SGPT.
- 2. ALT is stable in serum for 3 days at 2° 8° C
- 3. Pyridoxal phosphate can elevate ALT values by activating the apoenzyme form of the transaminase. Pyridoxal phosphate may be found in water contaminated with microbial growth.

ASSAY PARA	ASSAY PARAMETERS:		
Mode	Kinetic.		
Wave Length	340nm.		
Reaction Slope	Decreasing.		
Temperature	37°C.		
Blank	Distilled Water.		
Reagents Volume	1000 ul.		
Sample Volume	100 ul		
Reaction time	180 sec		
Delay time	60 sec		
Delta time	60 sec		
Number of reading	3		
Factor	1745.		
Linearity	300 IU/L		
Units	IU/L.		

## **MANUAL ASSAY:**

Pipette into cuvettes	Macro	Semi-Micro
Reagent	800+200 ul	400+100 ul
Sample	100 ul	50ul

Mix and take the first reading after 60 sec, and take THREE additional readings at 60 sec, intervals Calculate mean absorbance change per minute (A/min).

#### **CALCULATIONS:**

 $ALT (IU / L) = A/min \times 1745.$ 

EXPECTED VALUES MEN

upto 42 IU/L

WOMEN : upto 32

IU/L

LINEARITY

: 300

IU/L

## DILUTION LIMIT:

The sample should be diluted 1 + 9 with 0.9% NaCl. Solution, if Abs. exceeds 0.17. Multiply the result by 10.

#### **REFERENCES:**

- 1. Henley, K.S, Pottard, H.M., J. Lab. Clin. Med. 46:785
- 2. Tietz, N.W., Fundamentals of Clinical Chemistry, W.B., Saunders Co., Phila, p682.
- 3. Henry, R.J., et al, Am. J. Clin. Path. 34:381.
- 4. Committee on Enzymes of the Scandinavian Society for clinical chemistry and clinical physiology, Scand. J. Clin. Lab. Invest. 32:291.