SGPT / ALT
UV Kinetic Method

DIATEK

CLINICAL SIGNIFICANCE :

Alanine Aminotransferase (ALT), also referred to as glutamate pyruvate transaminase (GPT), is an enzyme involved in amino acid metabolism. It is found in many tissues, but the highest levels are found in liver and kidney tissues. Tissues destruction leads to the release of the intracellular enzyme into the circulating blood. Markedly elevated serum ALT levels may be found in a variety of diseases which involve the liver, such as hepatitis, mononucleosis, and cirrhosis. These very high levels of ALT are not usually observed in other disease processes, e.g., myocardial infarction; thus, ALT is regarded as a reasonably specific indicator of liver disease.

TEST PRINCIPLE :

ALT present in the sample catalyzes the transfer of the amino group from L-alanine to -ketoglutarate, forming pyruvate and L-glutamate. Pyruvate in the presence of NADH and lactate dehydrogenase is reduced to L-lactate. In this reaction NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340nm due to the oxidation of NADH to NAD.

$$\text{L-alanine} + \alpha\text{-ketoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{L-glutamate}$$

$$\text{pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-lactate} + NAD^+ + H_2O$$

REAGENTS COMPOSITION :

Working Reagent (R1 + R2)

NADH	0.18mmol/L
ketoglutarate	15mmol/L
L-alanine	500mmol/L
LDH	1200U/L
Tris Buffer(pH=7.50)	100mmol/L

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8 ºC, and contaminations prevented during their use. Do not use reagents over the expiration date.

KIT CONTENTS

CODE No.	*PT01*	*PT02*	*PT03*
Pack size :	(5 x 10 ml)	(5 x 25 ml)	(5 x 100 ml)
Reagent 1	5 x 8 ml	5 x 20 ml	5 x 80 ml
Reagent 2	1 x 10 ml	1 x 25 ml	1 x 100 ml

REAGENT PREPARATION

Working Reagent is prepared by mixing 4 part of Reagent 1 with 1 part of Reagent 2. The working reagent is stable upto 28 days at 2 - 8 ºC . It is recommended to use by prepare of a fresh WR based on its workload.

SAMPLES : Serum or Heparinised / EDTA Plasma

Serum should be separated from blood as soon as possible.

INTERFERENCES

Gross haemolysed and lipemic sample should not be used
The following analytes were tested up to the levels indicated and found not to interfere Hb 500 mg/dl, TG 1000 mg/dl Bilirubin (Total) 40 mg/dl, Ascorbic Acid: 50 mg/dl

ASSAY CONDITIONS:

Wavelength :	340 nm
Cuvette:	1 cm light path
Constant temperature	37ºC
Reaction (Mode)	Kinetic
Kinetic Factor	1746
Delay	60 sec
Read time	180 sec
Linearity	400 IU/L
Unit	IU/L
Blank	D. Water
Reaction Slope	Decreasing

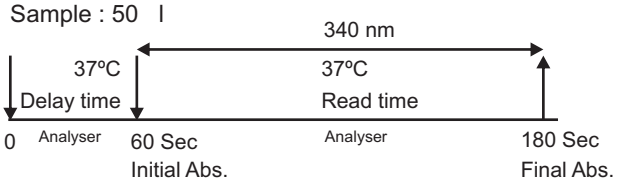
PROCEDURE :

1. Mix 50 µl sample with 500 µl Working Reagent, mix and read initial absorbance after 60 sec. and measure final absorbance after 180 sec against distilled water blank at 340 nm by an Analyser.

4. Calculate absorbance change per minute (△A/min).

Assay Procedure summary:

R1 : 400 l + R2 : 100 l
Sample : 50 l



CALCULATIONS :

SGPT (U/L) =△ Abs / mint x 1746

REFERENCE RANGE :

Upto 42 IU/L

The above reference range is guideline and all the laboratories must establish their own normal reference range. Final diagnosis should be made with correlation of clinical factors.

PRECAUTIONS :

1. Storage conditions as mentioned on the kit to be adhered.

2. Use clean glassware and microtips while pipetting Reagents

3. Avoid contamination of the reagent during the assay process.

4. Before the assay begins, bring all the reagents to room temperature.

5. If a larger volume of reagent is required for the absorbance reading, requisite volume can be taken in multiples, keeping the same ratio of reagent to specimen/standard.

6. Do not freeze or expose the reagents to high temperature and protect from direct sunlight as it will affect the performance of the kit.

7. Programmes for specific autoanalysers are available on request.

8. For accuracy of results, the assay procedure, reagent preparation and storage has to be meticulously followed.

9. As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

LINEARITY AND DETECTION LIMIT :

The assay is linear up to SGPT activity of 400 IU/L.
The results of the performance characteristics depend on the analyzer used. If the results obtained were greater than linearity limit, dilute the sample 1 : 10 with Normal Saline and multiply the result by 10.

BIBLIOGRAPHY

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