SGPT (ALAT) KIT

(Reitman & Frankel's method)

For the determination of SGPT (ALAT) activity in serum.

(For Invitro Diagnostic Use Only)

Summary

SGPT is found in a variety of tissues but is mainly found in the liver. Increased levels are found in hepatitis, cirrhosis, obstructive jaundice and other hepatic diseases. Slight elevation of the enzymes is also seen in myocardial infarction.

Principle

SGPT converts L-Alanine and α Ketoglutarate to Pyruvate and Glutamate. The Pyruvate formed reacts with 2,4,Dinitrophenyl hydrazine to produce a hydrazone derivative, which in an alkaline medium produces a brown coloured complex whose intensity is measured. The reaction does not obey Beer's law and hence a calibration curve is plotted using a Pyruvate standard. The activity of SGPT (ALAT) is read off this calibration curve.

L - Alanine + α Ketoglutarate —*SGPT, pH 7.4*→ **Pyruvate + L-Glutamate**

Pyruvate + 2,4,DNPH —*Alkaline Medium*→ **2,4,Dinitrophenyl Hydrazone (Brown coloured complex)**

Normal reference values

Serum = 5 - 35 Units/ml

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	40 Assays	80 Assays
L1 : Substrate Reagent	25 ml	50 ml
L2 : DNPH Reagent	25 ml	50 ml
L3 : NaOH Reagent (4N)	25 ml	50 ml
S : Pyruvate Standard (2 mM)	5 ml	5 ml

Storage / stability

Contents are stable at 2-8°C till the expiry mentioned on the labels. Sodium Hydroxide can be stored at R.T. till the expiry mentioned.

Reagent Preparation

All reagents are ready to use except NaOH Reagent (4N) which has to be diluted 1:10 with distilled/deionised water.

Working NaOH reagent : Dilute the Sodium Hydroxide to 250 ml or for every 1.0 ml of NaOH Reagent (4N) add 9.0 ml of water. The Working Sodium Hydroxide reagent is stable at R.T. till the expiry mentioned, in a **plastic bottle**.

Sample material

Serum. Free from hemolysis. SGPT (ALAT) is reported to be stable in serum for 3 days at 2-8°C.

Procedure

Wavelength / filter : 505 nm (Hg 546 nm) / Green
Temperature : 37°C & R.T.
Light path : 1 cm

Plotting of the Calibration curve :

Pipette into five clean dry test tubes labelled as 1, 2, 3, 4, & 5 :

Addition sequence	1	2	3	4	5
Enzyme Activity (U/ml)	0	28	57	97	150
	(ml)	(ml)	(ml)	(ml)	(ml)
Substrate Reagent (L1)	0.50	0.45	0.40	0.35	0.30
Pyruvate Standard (S)	-	0.05	0.10	0.15	0.20
Distilled Water	0.10	0.10	0.10	0.10	0.10
DNPH Reagent (L2)	0.50	0.50	0.50	0.50	0.50
Mix well and allow to stand at R.T. for 20 minutes.					
Working NaOH Reagent (L3)	5.0	05.0	05.0	05.00	5.00

Mix well and allow to stand at R.T. for 10 min. Measure the absorbances of the tubes 2 - 5 against tube 1 (Blank). Plot a graph of the absorbances of tubes 2 - 5 on the 'Y' axis versus the corresponding Enzyme activity on the 'X' axis.

Assay :

Pipette into clean dry test tubes labelled as Blank (B) & Test (T)

Addition Sequence	(B) (ml)	(T) (ml)
Substrate Reagent (L1)	0.50	0.50
Incubate at 37°C for 3 minutes		
Sample	-	0.10
Mix well and incubate at 37°C for 30 minutes		
DNPH Reagent (L2)	0.50	0.50
Mix well and allow to stand at R.T. for 20 minutes.		
Distilled Water	0.10	-
Working NaOH Reagent (L3)	5.00	5.00

Mix well and allow to stand at R.T. for 10 min. Measure the absorbances of the Test (T) against Blank (B) and read the activity of the test from the calibration curve plotted earlier.

Note

One sample blank is sufficient for each assay series.

If Enzyme activity exceeds 150 U / ml dilute the sample with distilled water and repeat the assay. Multiply the value with the proper dilution factor.

High concentrations of aldehydes and ketones in the sample or icteric or lipemic, samples may cause slightly elevated results. It is recommended to run a sample blank for these samples using serum instead of distilled water in the blank. High levels of serum pyruvate may interfere with the results.

References

Reitman, S., Frankel, S., (1957) Amer. J. Clin. Path. 28 : 56
Tietz, N.W., (1970) Fund. of Clin. Chem. P. 447

System Parameters

Reaction	:	End Point
Wavelength	:	505 nm
Zero Setting	:	Reagent Blank
Incub. Temp.	:	37°C
Incub. Time	:	50 min.
Delay Time	:	---
Read Time	:	---
No. of read.	:	---
Interval	:	---
Sample Vol.	:	0.10 ml
Reagent Vol.	:	6.00 ml
Standard	:	Calib. Curve
Factor	:	---
React. Slope	:	Increasing
Linearity	:	150 U/ml
Units	:	U/ml

GPT(R&F):02(P)

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