JOURI LABS

SGPT

Liquid Stable Reagent
End Point determination
Store at 2-8ºC

PRINCIPLE

Colorimetric determination of alanine Amino transferase based on the following reaction:

L - Alanine + oxoglutarate $\xrightarrow{\text{AIT}}$ Pyruvate + L.Glutamate

Pyruvate formed reacts with 2,4 dinitrophenyhydrazine to yield a colored hydrazone that can be measured at 546 nm (530 to 550 nm)

REFERENCE VALUES

Serum: 8 - 40 U/l

SAMPLES

Serum free of hemolysis separated from blood cells as soon as possible after collection.
Transaminase are stable in serum for 6h at 25-35°C, 7days at 2-8°C and for one month when stored at -20°C.

REAGENTS

R_1: GPT substate

phosphate buffer 7.4	100 mmol/l
L - alanine	200 mmol/l
2- Oxoglutarate	2.0 mmol/l

R_2 : GPT Color reagent

DNPH	1.0 mmol/l
HCl	1.0 mmol/l

R_3 : GPT Standard

Pyruvate

Materials Required but not Provided: NaOH 0.4N

The reagents are stable several years at 2-8°C
Reagent 2 should be stored in the dark.

PROCEDURE

Wavelength	546 nm (530-550nm)
Cuvette	1 cm light path
Zero adjustment	Distilled water
Method	Endpoint - Increasing

CALIBRATION CURVE
Pipette into test tubes (ml):

Tube No	Pyruvate R3	Substrate R1	Water	SGPT U/l
1	0.0	1.0	0.2	0
2	0.1	0.9	0.2	25
3	0.2	0.8	0.2	51
4	0.3	0.7	0.2	87
5	0.4	0.6	0.2	126

Add 1 ml dinitropheny hydrazine R2 mix let stand for 20 minutes at room temperature.
Add 10ml NaOH 0.4N, mix wait 5 min. Measure.
Plot the standard curve on millimeter paper:

- Abscissa: number of units/ml
- Ordinate: OD

The change in absorbance is not linearly related to the theoretical value of pyruvate produced and hence the enzyme activity.

MEASUREMENT:

	Sample
Sample	0.1ml
Solution 1	0.5 ml

Mix and let stand exactly 30 minutes at 37C.

Solution 2	0.5 ml

Mix and let stand exactly 20 minutes at 20 to 25C.

NaOH 0.4N	5 ml

Mix by gently inversion. Read absorbance against water after 5 minutes.
The color intensity is stable for 1 hour
Calculate the number of SGPT unites/l of serum using the standard curve

LINEARITY

If the concentration of sample exceeds 126 U/l dilute 0.1 ml of sample with 0.9ml of 0.9% sodium chloride solution.

NOTE

- Sample and reagents volumes may be altered proportionally to fit equipment requirement.
- Time and temperature are critical and must be observed any time you run the assay.

The following symbols are used on labels

IVD — **For in vitro diagnostic use**

Use day (last day of the month)

Temperature limitation

LOT — **Batch code**

REF — **Code**