

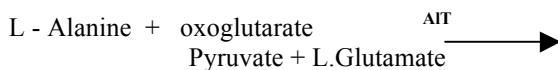


# SGPT

**Liquid Stable Reagent**  
**End Point determination**  
**Store at 2-8°C**

## PRINCIPLE

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



Pyruvate formed reacts with 2,4 dinitrophenylhydrazine 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<sub>1</sub>**: GPT substate

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

**R<sub>2</sub>** : GPT Color reagent

DNPH	1.0 mmol/l
HCl	1.0 mmol/l

**R<sub>3</sub>** : GPT Standard

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

NaOH 0.4N	5 ml
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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



For in vitro diagnostic use



Use day (last day of the month)



Temperature limitation



Batch code



Code