HiPer® SGPT (ALAT) Estimation Teaching Kit

Product Code: HTBC009
Number of experiments that can be performed: 20

Duration of Experiment: 2 hours 30 minutes

Storage Instructions:

- The kit is stable for 6 months from the date of manufacture
- Store Substrate Reagent (SGPT), Pyruvate standard (2mM), DNPH Reagent at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800

Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

Index

Aim:

To determine SGPT (ALAT) activity in serum by Reitman & Frankel Method.

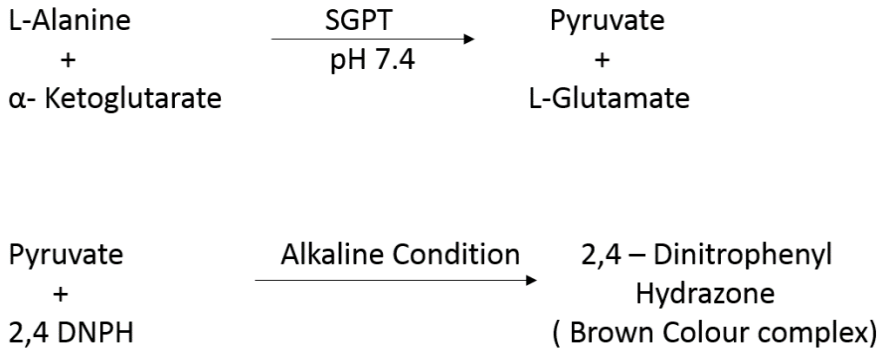
Introduction:

Serum glutamic pyruvic transaminase (SGPT) is also known as Alanine transaminases (ALT) or Alanine Amino Transferases (ALAT). The enzyme SGPT is widely reported in a variety of tissue sources. Alanine aminotransferase are enzymes found mainly in the liver, but also found in red blood cells, heart cells, muscle tissue and other organs, such as pancreas and kidneys. The major source of SGPT is of hepatic origin and has led to the application of SGPT determinations to study hepatic diseases. Elevated serum levels are found in hepatitis, cirrhosis, and obstructive jaundice. Levels of SGPT are only slightly elevated in patients following a myocardial infarction. when body tissue or an organ such as liver or heart is diseased or damaged, additional ALT are released into the bloodstream, causing levels of the enzyme to rise. Therefore, the amount of ALT in the blood is directly related to the extent of the tissue damage.

UV methods for SGPT determination were first developed by Wroblewski and LaDue in 1956. The method was based on the oxidation of NADH by lactate dehydrogenase (LDH). In 1957 Reitman and Frankel described a colorimetric method, and reagents for the procedure became available commercially. The method has become popular and widely used because of its simplicity and ease of performance.

Principle:

Glutamate pyruvate transaminases GPT or Alanine transaminases (ALAT) catalyzes the transfer of an amino group from 1-alanine to α-ketoglutarate resulting in the formation of pyruvate and L-glutamate respectively. Pyruvate so formed couples with 2,4 DNPH in an alkaline medium to form a brown coloured hydrazone complex. The intensity of the brown colour is proportional to SGPT (ALAT) activity and measured at 505nm.



Kit Contents:

Table 1: Enlists the materials provided in this kit for SGPT assay with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			20 expts	
1	TKC425	Substrate Reagent(SGPT)	84 ml	2-8 C
2	TKC422	Pyruvate standard (2mM)	12 ml	2-8°C
3	TKC423	DNPH Reagent	96 ml	2-8 C
4	TKC424	NaOH Reagent (4N)	96 ml	RT
5	ML064	Molecular Biology Grade Water	16 ml	RT

Materials Required But Not Provided:

Glasswares: 1 ml and 10 ml Pipettes, cuvettes, test tubes
Reagents: Distilled Water*
Other requirements: Spectrophotometer/Colorimeter, Micropipette and tips, test tube stand, Test serum sample, Incubator (37°C).
*Molecular biology grade water is recommended (Product code: ML064)

Storage:

HiPer® SGPT (ALAT) Estimation Teaching Kit stable for 6 months from the date of manufacture without showing any reduction in performance. Substrate reagent (SGPT), Pyruvate Standard and DNPH reagent can be stored at 2-8°C. Other kit contents can be stored at room temperature (15-25°C).

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. All glass wares must be clean and protein free, otherwise it will interfere with the assay.
3. The Test and standard samples should be treated identically for accurate results.
4. The assay should be carried out at the same time and in the same buffer conditions.
5. **Test Sample:** Serum sample free from hemolysis. SGPT (ALAT) is reported to be stable in serum for 3 days at 2-8°C.
6. **Preparation of 0.4N NaOH reagent:**
   To prepare 100 ml of 0.4N NaOH reagent, mix 10 ml of 4N NaOH with 90 ml of sterile distilled water.

Procedure:

Plotting of the standard curve:

1. Take five clean & dry test tubes and label them as 1, 2,3,4,5.
2. Prepare standards with Enzyme activity of 0,28,57,97,150 (U/ml) by transferring respective amount of reagents as mentioned in **Table 1**.
3. Mix well and allow to stand at RT for 20 minutes.
4. Add 5.0 ml of 0.4N NaOH reagent.
5. Keep at room temperature for 10 minutes.

Table 1

Tube No.	1 Blank	2	3	4	5
Enzyme Activity (U/ml)	0	28	57	97	150
SGPT Substrate Reagent (ml)	0.50	0.45	0.40	0.35	0.30
Pyruvate Standard (ml)	-	0.05	0.10	0.15	0.20
Distilled Water (ml)	0.10	0.10	0.10	0.10	0.10
DNPH Reagent (ml)	0.50	0.50	0.50	0.50	0.50
Mix well and allow to stand at R.T. for 20 minutes					
0.4N NaOH Reagent (ml)	5.0	5.0	5.0	5.0	5.0
Mix well and allow to stand at R.T. for 10 min.					
Measure the Absorbance of tube no 2-5 against tube 1(Blank) at 505 nm					

6. Switch on the Spectrophotometer, select the wavelength at 505 nm and let it warm before taking the absorbance (OD). Measure the Absorbance of Tube 2-5 against tube 1(Blank).
7. Remove Blank tube and take the OD of all the tubes and record it. Wash the cuvette after taking OD of each sample.
8. Plot a graph with absorbance of the tube 2-5 on Y- axis versus the corresponding enzyme activity on the X-axis.

Assay for Test sample:

1. Take two clean and dry test tubes labelled as Blank (B) and Test sample (TS).
2. Add 0.5 ml of Substrate reagent to each tube and incubate at 37°C for 3 minutes.
3. Add 0.1 ml of Distilled water to blank tube
4. Add 0.1 ml of test serum to test sample tube & Incubate both the tubes at 37°C for 30 minutes
5. Add 0.5 ml of DNPH reagent to all tubes. Mix well and allow it to stand for 20 minutes at RT.
6. Add 5.0 ml of 0.4N NaOH reagent. Mix well and allow it to stand at RT for 10 minutes.

Addition Sequence	Blank (B)	Test Sample (TS)
SGPT Substrate Reagent (ml)	0.5	0.5
Incubate at 37ºC for 3 minutes.		
Sample (ml)	-	0.1
Distilled water (ml)	0.1	-
Mix Well and Incubate at 37ºC for 30 minutes.		
DNPH Reagent (ml)	0.5	0.5
Mix well and allow to stand at RT for 20 minutes		
0.4N NaOH reagent (ml)	5.0	5.0
Mix well and allow to stand at RT for 10 minutes		
Absorbance of Test samples against Blank (B) at 505 nm		

7. Measure the absorbance of Test samples against Blank (B) at 505 nm and read the activity of the test from the standard curve.

Observation & Results:

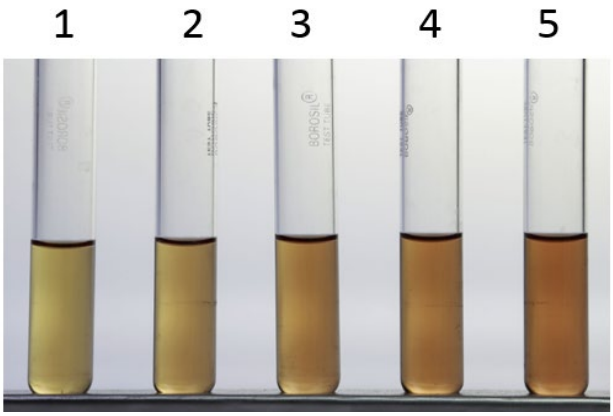
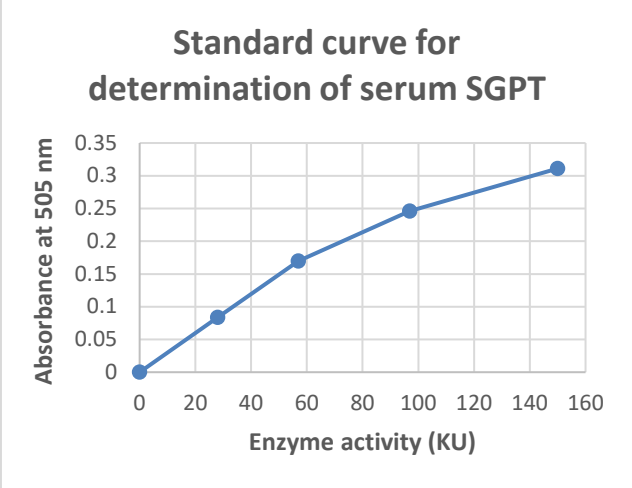


Fig 1: Determination of Serum SGPT- Intensity of brown colour increases with SGPT activity

Tube No	Standard samples
1	Blank
2	Standard 1
3	Standard 2
4	Standard 3
5	Standard 4

Determination of serum SGPT activity in Unknown Sample:

Draw a curve of Karmen enzyme unit on X-axis versus Absorbance at 505 nm on Y-axis and determine the concentration of SGPT in Test sample by extrapolating from absorbance value.



Interpretation:

The SGPT assay is carried out by preparing a set of solutions with known pyruvate standards and mixing them with the SGPT substrate reagent. A standard curve can be made and the concentrations of SGPT in Test serum sample can be derived from the standard curve.

Trouble shooting Guide:

Sr. No	Problem	Possible Cause	Solution
1	Standard and Test samples give lower OD values than expected although the Blank is ok	Procedure was not carried out properly	Follow the entire procedure carefully
		Absorbance was not measured at correct wavelength	Measure absorbance at correct wavelength as mentioned in the brochure

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of Technical assistance mail at mb@himedialabs.com

Symbol:

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIHTBC009
Rev No.: 03
Date of Issue: 2025-05