HiPer® SGOT (ASAT) Estimation Teaching Kit

Product Code: HTBC008
Number of experiments that can be performed: 20

Duration of Experiment: 3 hours

Storage Instructions:

- The kit is stable for 6 months from the date of manufacture
- Store Substrate Reagent (SGOT), 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

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Index

Sr. No.	Contents	Page No.
1	Aim	3
2	Introduction	3
3	Principle	3
4	Kit Contents	3
5	Materials Required But Not Provided	4
6	Storage	4
7	Important Instructions	4
8	Procedure	4
9	Observation and Result	6
10	Interpretation	6
11	Troubleshooting Guide	7

Aim:

To determine SGOT (ASAT) activity in serum by Reitman & Frankel Method.

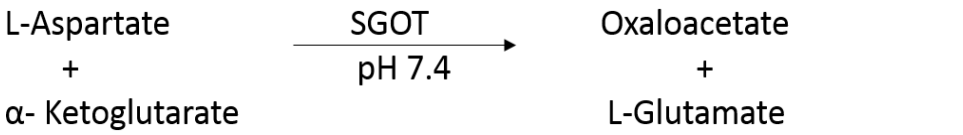
Introduction:

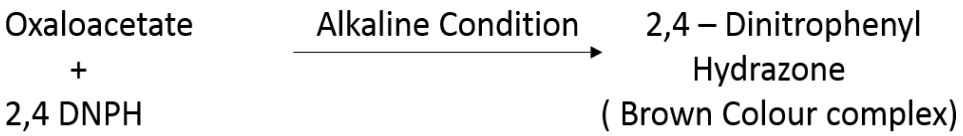
Serum Glutamic Oxaloacetate Transaminase (SGOT) also known as Aspartate Aminotransferase (AST/ ASAT) is a tissue enzyme that catalyzes the exchange of amino and keto groups between alpha-amino acids and alpha-keto acids. SGOT is widely distributed in tissue principally cardiac, hepatic, muscle and kidney. Injury to these tissues results in the release of the SGOT enzyme to general circulation. Following a myocardial infarction, serum levels of SGOT are elevated and reach a peak in 48 to 60 hours after onset. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma, and viral hepatitis also will increase serum SGOT levels. Decrease levels may be found in pregnancy, Beri-Beri and Diabetic ketoacidosis.

The first kinetic assay of SGOT for diagnostic purposes was described by Karmen et al. in 1955, using a coupled reaction of malate dehydrogenase (MDH) and NADH. In 1957 Reitman and Frankel described a colorimetric method. The method has become popular and widely used because of its simplicity and ease of performance.

Principle:

SGOT catalyses the transfer of Amino group from L-Aspartate to α-Ketoglutarate resulting in the formation of Oxaloacetate and Glutamate. The Oxaloacetate formed reacts with 2, 4-Dinitrophenyl hydrazine to produce a hydrazone derivative, which in an alkaline medium produces a brown colored complex whose intensity is measured colorimetrically at 505 nm.





Kit Contents:

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

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			20 expts	
1	TKC421	Substrate Reagent (SGOT)	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® SGOT (ASAT) Estimation Teaching Kit is stable for 6 months from the date of manufacture without showing any reduction in performance. Substrate reagent (SGOT), 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. SGOT (ASAT) 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*.

*Molecular Biology Grade Water is Recommended (Product code: ML064)

Procedure:

Plotting of the standard curve:

1. Take clean & dry five test tubes and label them as 1, 2,3,4,5.
2. Prepare standards with Enzyme activity of 0, 24, 61,114,190 (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. Mix well and allow it to stand at room temperature for 10 minutes.

Table 1

Tube No.	1 Blank	2	3	4	5
Enzyme Activity (U/ml)	0	24	61	114	190
SGOT 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 minutes					
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.

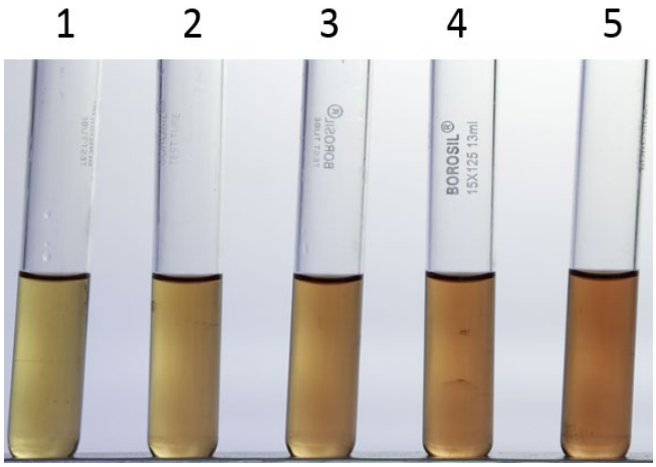
Assay for Test Sample:

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 60 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)
SGOT 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 60 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 sample against Blank (B) at 505 nm and read the activity of the test from the standard curve.

Observation & Results:

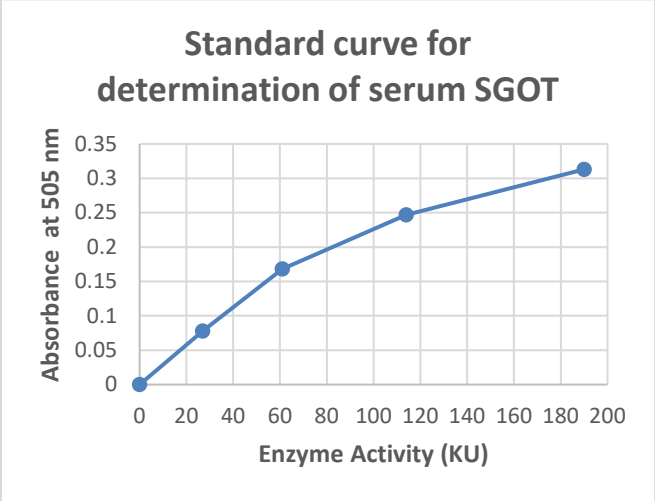


Tube No	Standard samples
1	Blank
2	Standard 1
3	Standard 2
4	Standard 3
5	Standard 4

Fig 1: Determination of Serum SGOT- Intensity of brown colour is proportional to SGOT activity

Determination of serum SGOT activity in Test Sample:

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



Interpretation:

The SGOT assay is carried out by preparing a set of solutions with known pyruvate standards and mixing them with the SGOT substrate reagent. A standard curve can be made and the concentrations of SGOT 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

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