SGPT (DST)

ENZOPAK

Last update 04-2014

(IFCC METHOD)

Reagent kit for quantitative estimation of glutamate pyruvate transaminase activity in serum or plasma.

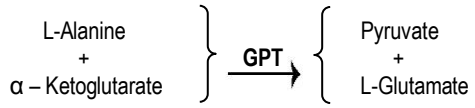
BACKGROUND & SYNOPSIS:

Wroblewsky and LaDue first devised a method for estimating glutamate pyruvate transaminase activity (also called alanine transminase, ALT). The primary transaminase reaction was coupled with lactate dehydrogenase and reduced nicotinamide adenine dinucleotide (NADH). This method was further improved by many workers and reviewed by professional societies like IFCC, GSCC, SCE etc.

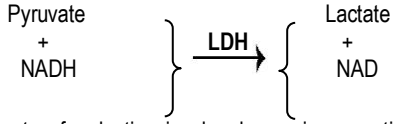
ENZOPAK SGPT is based on the procedure recommended by the IFCC.

PRINCIPLE:

- In this reaction, L-Alanine and alpha-ketoglutarate react in the presence of GPT in the sample to yield pyruvate and L-glutamate.



(a) Pyruvate is reduced by lactate dehydrogenase to yield lactate with the oxidation of NADH to NAD. The reaction is monitored by measurement of the decrease in absorbance of NADH at 340 nm.



The rate of reduction in absorbance is proportional to GPT activity in sample.

DIAGNOSTIC SIGNIFICANCE:

Alanine transaminase is present in high concentrations in liver, kidneys, heart and skeletal muscle tissue. It is also present in lungs, spleen, pancreas, brain and erythrocytes at a lower concentration. Primary liver diseases (cirrhosis, obstructive jaundice, carcinoma, viral or toxic hepatitis) as well as liver damage secondary to other causes result in elevated GPT levels. Patients undergoing extended hemodialysis without supplemental vitamin B_6 therapy may show low GPT in serum.

REAGENT COMPOSITION :

Active Ingredients	**Concentration**
Reagent-1	
• NADH Na_2	0.1 mmol/L
• LDH	2000 U/L
Reagent-2	
• Buffer	50 mmol/L
• L-Alanine	200 mmol/L
• α–KG	10 mmol/L

pH 7.5±0.1 at 25^{0}C

Also contains non-reactive fillers and Stabilizers.

PRESENTATION:

All Reagents: Store at 2-8^{0}C

Pack Size	**No. of Bottles/Vials**	
	1 SGPT (Enzyme/Coenzyme)	2 SGPT (Buffer Substrate)
20 x 1.1 ml	(2x10 Tablets)	2
5 x 10 ml	5	1
5 x 20 ml	5	5
4 x 50 ml	4	4

(For 20x 1.1ml & 5x10 ml Reconstitution Bottle Provided)

CE

An ISO 13485: 2012 & GMP Certified Company

PRECAUTION:

ENZOPAK SGPT is for *IN-VITRO* diagnostic use only.

Reagent contains Sodium Azide, DO NOT INGEST.

WORKING REAGENT PREPARATION:

FOR 20 x 1.1 ml.:

Add 1.1 ml. of 2 SGPT to one tablet of 1 SGPT. Mix well to dissolve and wait for 15 minutes prior to use. The working reagent is stable for 30 days at 2-8^{0}C.

FOR 5 x 10 ml. :

Carefully transfer the content of 1 SGPT (powder) into the bottle containing 10 ml of 2 SGPT (buffer). Mix gently to dissolve completely. Wait for 5 minutes before use. The working reagent is stable for 4 months at 2-8^{0}C.

FOR 5 x 20 ml. :

Carefully transfer the content of 1 SGPT (powder) into the bottle containing 20 ml of 2 SGPT (buffer). Mix gently to dissolve completely. Wait for 5 minutes before use. The working reagent is stable for 4 months at 2-8^{0}C.

FOR 4 x 50 ml. :

Carefully transfer the content of 1 SGPT (powder) into the bottle containing 50 ml of 2 SGPT (buffer). Mix gently to dissolve completely. Wait for 5 minutes before use. The working reagent is stable for 4 months at 2-8^{0}C.

REAGENT STORAGE AND STABILITY:

ENZOPAK SGPT reagents are stable at 2-8^{0}C until the expiry date stated on the label.

SPECIMEN COLLECTION:

Fresh, clear serum under fasting condition with no hemolysis is the specimen of choice. Plasma collected with anticoagulants such as heparin or EDTA may be used.

PROCEDURE:

REACTION PARAMETERS

• Type of Reaction	:	Kinetic / Decreasing
• Wavelength	:	340 nm
• Flowcell Temperature	:	37^{0}C
• Delay Time	:	60 seconds
• Interval	:	30 seconds
• No. of Readings	:	4
• Sample volume	:	100 µl (0.1 ml)
• Working reagent	:	1.0 ml
• Factor	:	1746
• Light Path	:	1 cm.
• Zero setting with	:	Distilled water

PIPETTE INTO TEST TUBES	**TEST**
• WORKING REAGENT (ml)	1.0
• SAMPLE (ml)	0.1

Mix and after incubation at 37^{0}C for 60 seconds, measure the absorbance at an interval of 30 seconds for 2 minutes at 340 nm.

NOTE:
For laboratories using instrument with cuvette capacity less than 1 ml, decrease the sample and working reagent volumes proportionately.

TEST RESULTS:
Serum GPT activity (IU/L) = ΔA/min. x F
Where = 1746 (based on the millimolar Extinction coefficient of NADH at 340 nm).

NORMAL VALUES :
GPT: 5-55 IU/L

LINEARITY:
The method is linear upto 500 IU/L. For sample values higher than 500 IU/L, dilute the sample suitably with 0.9% saline and repeat the assay.
Apply the dilution factor to calculate the final results.

REFERENCES:

- The Committee on Enzymes of the Scandinavian Society for Clini cal Chemistry and Clinical Physiology, Recommended methods for determination of four enzymes in blood, Scan J. Clin. Lab. In vest 33, 291 (1974).

- HENRY, R.J., CHIAMORI, M., GOLUB O.J. and BERKMAN, S., Revised spectrophotometric methods for the determination of glutamic oxaloacetic transaminase, glutamic pyruvate transami nase and lactic acid dehydrogenase, Am. J. Clin. Pathol. 34, 381-398 (1960).