ENZOPAK

Last update 04-2014

SGPT (ALT) LIQUID

(IFCC METHOD)

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

- Long shelf life.
- Liquid stable reagents available.
- International Standard 'IFCC' reagent.

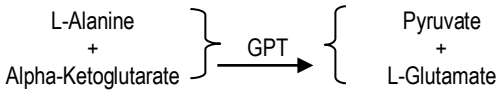
BACKGROUND & SYNOPSIS:

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

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

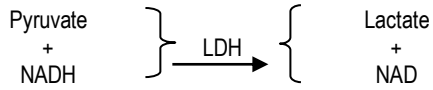
PRINCIPLE:

1) In this reaction, L-alanine and alpha-ketoglutarate react



in the presence of GPT with the sample to yield pyruvate and L-glutamate.

2) 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 SGPT 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 lung, 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 B6 therapy may show low GPT in serum.

REAGENT COMPOSITION

Active Ingredients	**Concentration**
Reagent-1	
• Buffer pH 7.6 ± 0.1	100 mmol/L
• LDH (Microbial)	> 2000 U/L
• L-Alanine	440 mmol/L
Reagent-2	
• 2-oxoglutrate	14 mmol/L
• NADH	0.1 mmol/L

Also contains non-reactive fillers and stabilizers.

CE

PRESENTATION:

Pack size	**1x25ml**	**2x25ml**
All reagents to be stored at 2-8^{0}C		
• SGPT – L (R1) (Enzyme reagent)	1x20ml	2x20ml
• SGPT – L (R2) (Substrate reagent)	1x5ml	2x5ml

PRECAUTIONS:

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

Reagent contains Sodium Azide. DO NOT INGEST.

WORKING REAGENT PREPARATION:

FOR 1x10ml, 1x25ml & 2x25 ml:

Carefully transfer the contents of 1 bottle of R2 into the bottle of R1. Mix well. Wait for 2 minutes before use.

Alternatively for flexibility as much of working reagent may be made as and when desired by mixing 4 parts of R1 & 1 part of R2.

Alternatively 0.8 ml of R1 and 0.2 ml of R2 may also be used instead of 1 ml of working reagent directly during the assay.

REAGENT STORAGE AND STABILITY :

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

The working reagent is stable for 30 days at 2-8^{0}C

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 can also be used.

PROCEDURE :

REACTION PARAMETERS

- Type of reaction : Kinetic/Decreasing OD
- Wavelength : 340 nm
- Flow cell Temperature : 37^{0}C
- Delay Time : 60 Seconds
- Interval : 30 seconds
- No. of Intervals : 4
- Sample Volume : 50 µl (0.05 ml)
- Working Reagent Volume : 1.0 ml
- Factor : 3376
- Light Path : 1 cm
- Zero setting with : Distilled Water

One Reagent Procedure

PIPETTE INTO TEST TUBES	**TEST**
• Working Reagent (ml)	1.0
• Sample (ml)	0.05

Two Reagent Procedure

PIPETTE INTO TEST TUBES	**TEST**
• SGPT - L R1 (ml)	0.8
• SGPT - L R2 (ml)	0.2
• Sample (ml)	0.05

Mix well 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.

SGPT (ALT) LIQUID

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 F = 3376 (based on the milimolar extinction coefficient of NADH at 340 nm).

NORMAL VALUES:
SGPT : 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 Clinical Chemistry & Clinical Physiology, Recommended methods for determination of four enzymes in blood, Scan J. Clin. Lab. Invest 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 transminase and lactic acid dehydrogenase, Am. J. Clin. Pathol.34, 381-398 (1960).

3/7,B.I.D.C., Gorwa, Vadodara 390 016 (INDIA)
Web: www.reckondiagnostics.com
Phone: +91·265·2281631
Email: mail@reckondiagnostics.com

RECKON DIAGNOSTICS P. LTD.