

ALT (SGPT) REAGENT (COLORIMETRIC, ENDPOINT METHOD) Catalog Number: BQ 004A-CR

INTENDED USE

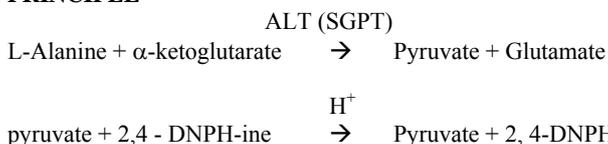
Alanine Aminotransferase (ALT) reagent is used for the quantitative determination of alanine aminotransferase (Glutamate pyruvate transaminase, SGPT) in human serum.

INTRODUCTION

The enzyme alanine aminotransferase is widely reported in a variety of tissue sources. The major source of ALT (SGPT) is of hepatic origin and has led to the application of ALT (SGPT) determinations in the study of hepatic diseases. Elevated serum ALT (SGPT) levels are found in hepatitis, cirrhosis, and obstructive jaundice. Levels of ALT (SGPT) are only slightly elevated in patients following a myocardial infarction.¹

Since 1955 many methods and modifications have been proposed for the determination of ALT (SGPT). The various methods generally fall into two categories: colorimetric and ultraviolet. It is generally agreed that the ultraviolet method is more sensitive than the colorimetric method. Our colorimetric method is based on dinitrophenylhydrazine formation. This method is relatively simple and has limited but acceptable accuracy.^{2,3}

PRINCIPLE



The method used here is a modification of the classical Reitman Frankel colorimetric endpoint reaction.⁴ In this procedure ALT (SGPT) catalyzes L-alanine and α -ketoglutarate to form pyruvate and glutamate. The pyruvate is then reacted with 2,4-dinitrophenylhydrazine (2,4-DNPH-one) to form 2,4-DNPH-one. The addition of sodium hydroxide dissolves this complex, allows 2, 4-DNPH-one to be measured at 505 nm.

REAGENTS

- ALT (SGPT) SUBSTRATE:** 0.2 M L-alanine, 2.0 mM α -ketoglutarate, 100 mM phosphate buffer at pH 7.4 + 0.05, 0.2% v/v preservatives.
- ALT (SGPT) COLOR REAGENT:** 1.0mM 2, 4 - dinitrophenylhydrazine in 1N Hydrochloric Acid, preservative. **CAUSES BURNS!**
- ALT (SGPT) Color Developer:** 0.5N sodium hydroxide. **CORROSIVE!**
- ALT (SGPT) CALIBRATOR:** Solution of sodium pyruvate in 100 mM phosphate buffer at pH 7.4. The activity will be provided in each lot.

REAGENT PREPARATION

All reagents are ready to use.

REAGENT STORAGE AND STABILITY

Store ALT (SGPT) Substrate, Color Reagent, Color Developer, and Calibrator at 2 – 8 °C. All the reagents are stable until the expiration date stated on the label.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.
CAUTION: In vitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures, which dictate avoiding ingestion and eye or skin contact.
- Specimens should be considered infectious and handled with care.
- ALT (SGPT) Color Reagent contains 1N hydrochloric acid, which causes BURNS. In case of contact, flush affected area with large amounts of water. Seek medical attention.
- ALT (SGPT) Color Developer contains 0.5N sodium hydroxide, which is CORROSIVE. In case of contact, flush affected area with large amounts of water. Seek medical attention.
- Exercise the normal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent.

REAGENT DETERIORATION

- Turbidity and precipitation have occurred; these may be signs of reagent deterioration.
- The reagent fails to meet linearity claims or fails to recover control values in the stated range.

SPECIMEN COLLECTION

This assay is intended for use with serum. Reports indicate that ALT (SGPT) in serum remains stable at 4 – 8 °C for a minimum of seven (7) days.⁵ Hemolyzed specimens should not be used as erythrocytes contain fifteen times the ALT (SGPT) activity in serum.⁴

INTERFERING SUBSTANCES

Pyridoxal phosphate can elevate ALT (SGPT) values by activating the apoenzyme form of the transaminase.⁶ Pyridoxal phosphate may be found in diluent water contaminated with microbial growth.

High levels of serum pyruvate may also interfere with assay performance. Young *et al.* give a list of drugs and other substances that interfere with the determination of ALT (SGPT) activity.⁷

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipetting devices
- Test tubes/rack
- Timer
- Spectrophotometer with capability to read at 505 nm
- Heating block or bath (37 °C)

MANUAL ENDPOINT PROCEDURE

- Label test tubes "Blank", "Calibrator", "Control", "unknown" etc.
- Transfer 0.5 ml of ALT (SGPT) substrate to each tube and place in a 37°C heating bath for 3 - 5 minutes.
- At timed intervals (about 15 - 30 seconds), add 0.1 ml (100 μ l) of sample to the correspondingly labeled tube. Mix and immediately return to 37°C heating bath for exactly 30 minutes.
- After exactly 30 minutes, add 0.5 ml of ALT (SGPT) Color Reagent to each tube, maintaining the timed interval sequence. Mix and return to 37 °C heating bath for exactly 10 minutes.
- After exactly ten (10) minutes, add 2.0 ml of ALT (SGPT) Color Developer (maintaining the same timed intervals). Mix and return to 37°C heating bath for five (5) minutes.
- Zero the spectrophotometer with the reagent "blank" at 505 nm. Read and record absorbance of all tubes. (Wavelength range: 500 – 520 nm).

* MULTI-PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.

NOTES:

- The final color produced in the reaction should be measured within 60 minutes.
- If the sample is icteric or lipemic, a serum blank must be run.
- If the ALT (SGPT) values exceed 120 IU/L, it is recommended that the test be repeated using a 1:4 dilution of the sample with 0.9% saline. The result is then multiplied by 5, the dilution factor.

CALCULATIONS

Use the absorbance reading of the calibrator and unknown(s) to calculate ALT (SGPT)

$$\frac{\text{Abs. of unknown values}}{\text{Abs. of calibrator of unknown (IU/L)}} \times \text{Conc. of calibrator (IU/L)}$$

= ALT (SGPT) value of unknown (IU/L)

Example:

Abs. (unknown) = 0.080

Abs. (calibrator) = 0.180

ALT (SGPT) concentration of calibrator = 70 IU/L

$$\frac{0.080}{0.180} \times 70 = 31 \text{ IU/L}$$

QUALITY CONTROL

Normal and abnormal control sera of known concentrations of ALT (SGPT) should be analyzed routinely with each group of unknown samples.

EXPECTED VALUES⁴

5 - 35 IU/L.

It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

PERFORMANCE CHARACTERISTICS

1. Linearity: 120 IU/L.
2. Sensitivity: Based on an instrument resolution of 0.001 absorbance, the present procedure has a sensitivity of 0.5 IU/L.
3. Comparison: A comparison studies between the present method with a similar method yield a correlation coefficient of 0.97 and a regression equation of $y = 0.99x + 0.71$.
4. Precision:

Run-to-Run precision was obtained by assaying two commercial control sera representing normal and abnormal results for a period of 30 days.

	<u>Mean(IU/L)</u>	<u>S.D.</u>	<u>C.V. %</u>
Normal	21.0	2.7	12.8
Abnormal	100.3	2.4	2.3

Within run precision was obtained by assaying two commercial control sera representing normal and abnormal results for twenty (20) times.

	<u>Mean(IU/L)</u>	<u>S.D.</u>	<u>C.V. %</u>
Normal	22.3	2.5	11.2
Abnormal	101.0	2.6	2.5

REFERENCES

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3. Winsten, S., et al.: *Clin. Chem.* 15:496 (1969).
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5. Henry, R.J.: *Clinical Chemistry Principles and Techniques*. 2nd Ed. Harper and Row, New York, NY p. 882 (1974).
6. Rej. R., et al.: *Clin. Chem.* 19:92 (1973).
7. Young, D.S., et al.: *Clin. Chem.* 21:5 (1975).