

Experiment no.: 6.

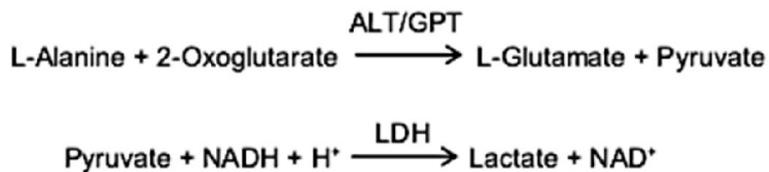
Experiment name: Determination of ALT/GPT in the blood serum.

The aim of the Experiment:

Determination of ALT/GPT BR in the blood using IFCC *UV enzymatic method* KINETIC.

Alanine aminotransferase (ALT/GPT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate. The latter is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH).

The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD⁺, proportional to the activity of ALT present in the sample.



Equipment and materials used in the Experiment:

- Photometer or spectrophotometer with a thermostatted cell compartment set at 30/37 °C, capable of reading at 340 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

REAGENT COMPOSITION

R1 ALT substrate. TRIS buffer 150 mmol/L pH 7.3, L-alanine 750 mmol/L, lactate dehydrogenase > 1350 U/L.

R2 ALT coenzyme. NADH 1.3 mmol/L, 2-oxoglutarate 75 mmol/L. Biocides.

Property of the machine:

Normal UV-Vis spectrophotometer:

Machine usage:

- 4- Wavelength set up step.

5- Blank against the solvent solution using a proper cuvette.

6- Reach O.D.

Experiment procedure or protocol:

PROCEDURE		
Preincubate working reagent, samples and controls to reaction temperature. Set the photometer to 0 absorbance with distilled water. Pipette into a cuvette:		
Reaction temperature	37 °C	30 °C
Working reagent Sample	1.0 mL 50 µL	1.0 mL 100 µL
4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.		
5. Incubate for 1 minute and record initial absorbance reading.		
6. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.		
7. Calculate the difference between absorbances.		
8. Calculate the mean of the results to obtain the average change in absorbance per minute ($\Delta A/\text{min}$).		

Experiment data and results:

Adults	37 °C	up to 40 U/L (0.67 µkat/L)
	30 °C	up to 25 U/L (0.42 µkat/L)

Levels approximately twice the adult level are seen in neonates and infants; these decline to adult level by approximately 6 months of age. It is recommended that each laboratory establishes its own reference range.

Conclusion:

- Where is GPT synthesized from?
- How does it affect the health?
- What are the normal level values?
- How can you determine its quantity on the blood?
- Discuss the methodology?