SGPT - ALT

Optimised IFCC Method

INTENDED USE:

This reagent kit is intended for "in vitro" quantitative determination of SGPT-(ALT) activity in serum/ plasma.

CLINICAL SIGNIFICANCE:

ALT is found in the cytosol of cells, it is a non-tissue specific soluble enzyme. ALT catalysis the transfer of amino groups during the transformations of aminoacids and alpha-ketoacids. Pyridoxal phosphate activates the process. The enzyme found in the serum is principally derived from the liver and kidney. The serum enzyme activity is increased during various hepatic disease states including hepatitis.

PRINCIPLE:

ALT catalysis the transformation of L-Alanin and 2-Oxoglutarate at optimal pH. The Pyruvate released in the reaction is transformed by Lactate dehydrogenase (LDH) in the presence of NADH /NAD* coenzyme to L-lactate, while the NADH/NAD* oxidoreductive process shows a decrease in absorbance at 340 nm. The change in absorbance correlates withserum ALT activity.

REAGENT COMPOSITION:

Reagent 1: Enzyme Reagent
Reagent 2: Substrate Reagent

MATERIALS REQUIRED BUT NOT PROVIDED:

- Clean & Dry Glassware.
- Micropipettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

SAMPLES:

Serum free of hemolysis. Heparin or EDTA plasma.

WORKING REAGENT PREPARATION & STABILITY:

Mix 4 Volume of Reagent 1, with 1 Volume of Reagent 2.
Working Reagent is stable for 30 days at 2-8°C.

GENERAL SYSTEM PARAMETERS:

Reaction type	Kinetic Reaction (Decreasing)
Wave length	340 nm
Light Path	1 Cm
Reaction Temperature	37°C
Blank / Zero Setting	With Distilled Water
Reagent Volume	1ml
Sample Volume	100 µ.
Lag/Delay Time	60 Sec.
Read Time	180 Sec.
Interval Time	60 Sec.
Factor	1746
Low Normal at 37°C	O U/I
High Normal at 37°C	40 U/I
Linearity	300 U/I
Reagent Absorbance Limit	>0.8
Max. Δ Abs/Min	0.171

ASSAY PROCEDURE:

Working Reagent	1000 µl
Sample	100 µl

Mix and after 60 second incubation, measure the decrease in absorbance every minute during 3 minutes at 37°C.
Determine the ΔA/min.

CALCULATION:

At 340 nm with 1cm Light path
SGPT Activity (U/l) = ΔA/min. x 1746

LINEARITY:

Reagent is Linear up to 300 U/l
Dilute the sample appropriately and re-assay if SGPT Activity exceeds 300 U/l or Δ Abs/min Exceeds 0.171. Multiply result with dilution factor.

REFERENCE NORMAL VALUE:

0 to 40 U/l
Normal Values for infants are higher than adults.
The reference values are only indicative in nature. Every laboratory should establish its own normal ranges.

QUALITY CONTROL:

For accuracy it is necessary to run known controls with every assay..

LIMITATION & PRECAUTIONS:

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
3. Before the assay bring all the reagents to room temperature.
4. Avoid contamination of the reagent during assay process.
5. Use clean glassware free from dust or debris.
6. Reagent to sample ratio as mentioned here above must be strictly observed as any change in to it will effect the factor.
7. Higher ALT/GPT values may induce falsely low result due to depletion of the substrate (total consumption of NADH before reading of the result). If an analyzer is used verify the presence of depletion factors on application.

BIBLIOGRAPHY:

Expert Panel on enzyme of the IFCC, Clin. Chem. Acta, 70, PM, (1976), Teitz., N.W.

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