**Determination of ALT (Alanine aminotransferase) in human serum**

Theory: Aminotransferases are enzymes that facilitate the conversion of one amino acid to another. This helps maintain a balanced supply of amino acid units needed for protein synthesis. Increased alanine aminotransferase activity is an important indicator of liver, heart and skeletal muscle activity.

In practice, transaminases are the body's own substances, which are usually found in cells. ALT transaminase is found mainly in the cells of the liver, heart, skeletal muscles, kidneys, brain and red blood cells. After their breakdown, they pass into the blood serum. Thus, increased ALT means increased cell lysis in these areas.

Standard: 0.06 - 0.14 ukat / l

Limit value: 0.42 ukat / l

Task: To determine ALT in human serum

Accessories: eppenndorph stand

 adjustable pipettes

 thermal bath at 37oC

 ELISA reader with 340 nm filter

Principle of the method: alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase EC2.6.1.2) catalyzes the reaction between L-alanine and 2-oxoglutarate, which converts to L-glutamate and pyruvic acid in an alkaline environment. Pyruvic acid hydrazone has a higher absorbance.

L-alanine + oxoglutarate pyruvate + L-glutamate

Pyruvate + NADH + H +  lactate + NAD +

The catalytic concentration of ALT is proportional to the decrease in absorbance at 340 nm.

Reagents

R1. Buffer: Tris buffer pH = 7.5, L-alanine, LD

LD ≥ 2.5 cat

NADH 21.6 /mol / vial

R2. Starter

NADH, 2-oxoglutarate 180 mmol / l

Sodium azide 0.1%

Activator

Pyridoxal-5-phosphate 6 µmol / tablet

Calibration

BIO-LA-TEST LYONORM CALIBRATOR, cat. No. (1,40µkat / l), 3204,3206

Preparation of working solution

Initially, the contents of the Reagent 1 vial are dissolved in 100 ml of Reagent 3. After dissolution, 2 tablets of Reagent 4 are added.

Adjusted to: 25% by weight of the contents of the Reagent 1 vial are dissolved in 25 ml of Reagent 3 solution.

Analysis procedure

Samples: non-hemolytic serum, heparinized or EDTA plasma

Wavelength: 340 nm

ELISA plate

Temperature: 37 ° C

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample type | amount | Working solution | 10min inkubation | Reagent  2 |
| Serum sample 2x diluted | 10 µl | 100 µl |  | 10 µl |
| Blank (Fyz. roztok) | 10 µl | 100 µl |  | 10 µl |
| Standard 2x diluted | 10 μl | 100 µl |  | 10 µl |
| Standard concentrated | 10 μl | 100 µl |  | 10 µl |

Use a blank, use Lyonorm (biochemical) as a standard

Mix and incubate at 37 ° C for 10 minutes

Reagent 2 is added in an amount of 10µl

Mix, incubate for 2 minutes at 37 ° C, measure absorbance at 1 minute intervals for at least 3 minutes. Calculate the average change in absorbance over 1 min (δA).

δA = average (A1 + A2 + A3)