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 37°C
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)