



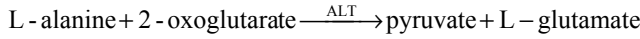
ALT (GPT)
Alanine aminotransferase
E.C.2.6.1.2.
Colorimetric Method

Cat.No. 101-0026

Size 200 tests

PRINCIPLE:

The enzyme ALT catalyzes the following reaction:



The pyruvate in reaction with 2,4-dinitro-phenylhydrazine forms pyruvate hydrazones which are brown in alkaline medium. The product is determined photometrically at 505 nm.

SAMPLE:

Serum (nonhemolyzed).

REAGENTS:

- | | |
|-------------------------------------|------------|
| 1. Buffer-substrate (1 x 100 ml) | |
| Triethanolamine-EDTA buffer, pH 7.5 | 50 mmol/L |
| L-alanine | 200 mmol/L |
| 2-oxoglutarate | 2 mmol/L |
| | |
| 2. Color reagent (1 x 100 ml) | |
| 2,4-dinitrophenylhydrazine (DNPH) | 1 mmol/L |
| | |
| 3. Standard (1 x 10 ml) | |
| Sodium pyruvate | 2 mmol/L |
| | |
| 4. Additional reagent | |
| NaOH (Cat. No. 101-0023) | 0.4 mol/L |

All reagents to be used undiluted.

Reagents are stable up to the expiry date when stored at +2 °C to +8 °C

PROCEDURE:

- | | |
|------------------|------------------|
| Wavelength: | 505 nm (490-520) |
| Cuvette: | 1 cm light path |
| Temperature: | 37 °C |
| Color stability: | 60 min. |
| Zero: | Reagent blank |

| | | |
|--|--------|---------------|
| Pipette into test tubes: | Sample | Reagent blank |
| Buffer-substrate | 0.5 ml | 0.5 ml |
| Sample | 0.1 ml | - |
| Distilled water | - | 0.1 ml |
| Mix and incubate for exactly 30 min. at 37 °C. | | |
| DNPH | 0.5 ml | 0.5 ml |
| Mix well and let stand for exactly 20 min. at 20 to 25 °C. | | |
| NaOH | 5.0 ml | 5.0 ml |
| Mix and after 5 minutes read the absorbance of sample against the reagent blank. | | |

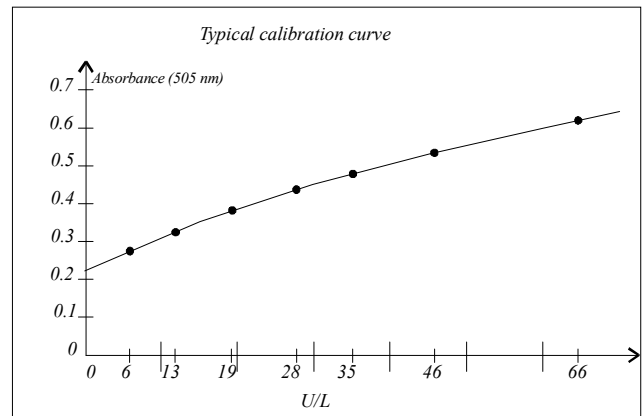
CALCULATION:

Using the absorbance values of the samples, read off the enzyme activity in U/L from calibration curve.

PREPARATION OF CALIBRATION CURVE:

| Pipette into tubes: | Test tube no. | | | | | | | | |
|---|---------------|------|------|------|------|------|------|------|------|
| | Blank | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Redistilled water | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Buffer-substrat | 1.00 | 0.95 | 0.90 | 0.85 | 0.80 | 0.75 | 0.70 | 0.60 | 0.50 |
| Standard | - | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 | 0.30 | 0.40 | 0.50 |
| DNPH | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Mix and let stand for 20 minutes at 20°C to 25°C | | | | | | | | | |
| 0.4 mol/L NaOH | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Mix and after 5 minutes measure the absorbance of each solution in test tubes 1 to 8 against the reagent blank at 505 nm (or green filter). | | | | | | | | | |
| ALT U/L | 0 | 6 | 13 | 19 | 28 | 35 | 46 | 66 | 99 |

Plot (millimeter paper) the absorbance values determined on the ordinate against the U/L values (from the table) on the abscissa and use these points draw a calibration curve.



EXPECTED VALUES:

ALT up to 17 U/L (283 nkat/L)

U/L x 16.67 = nkat/L

LINEARITY:

up to 50 U/L (833 nkat/L)

QUALITY CONTROL:

All commercial control sera with established values for this method.

NOTE:

- If the activity is higher than 80 U/L, dilute sample 1:5 with saline and multiply the result by 5.
- Buffer-substrate and standard contain sodium azide. Avoid ingestion or contact with skin or mucous membranes.

REFERENCES:

- Reitman, S., and Frankel, S., Amer. J. Clin. Path., 1957;28:56
- Bergmeyer H.U., Clin. Chem. 1972;18:1305