Reactivos GPL

Barcelona, España

CE

- GPT(ALT)-LQ - GPT/ALT
LDH-NADH. Cinetico UV. Líquido

Store at: +2+8ºC.

Presentation:
Cod. EZ016LQ CONT: R1 1 x 100 R2 1 x 25 mL .
EZ017LQ CONT: R1 2 x 100 R2 2 x 25 mL .

Procedure

Quantitative determination of alanine aminotransferase GPT /ALT.

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY
Alanine aminotranferase (ALT) o Glutamate pyruvate transaminase (GPT) catalyses the reversible transfer of an amino group from alanine to α-ketoglutarate forming glutamate and piruvate.
The piruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH:

$$\text{Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Glutamate} + \text{Piruvate}$$

$$\text{Piruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic concentration of ALT present in the sample[1].

COMPOSICIÓN DE LOS REACTIVOS

R.1 (Buffer)	TRIS PH 7.8 L-Alanine Lactate dehydrogenase (LDH)	100 mmol/L. 500 mmol/L. 1200 U/L.
R.2 (Substrate)	NADH α-Ketoglutarate	0.18 mmol/L. 15 mmol/L.

REAGENT PREPARATION AND STABILITY
Working reagent (WR):
Mix 1 volume of R2 with 4 volumes of R1.
Stability: 21 days at 2-8ºC or 72 hours at room temperature.
All the components of the kit are stable until the expiration date on the label when stored at 2-8ºC, protected from light and contamination prevented during their use.
Do not use reagents over the expiration date.

Signs of Reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm. < 1.00

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8ºC,. Do not use reagents over the expiration date.

SPECIMEN
Serum or plasma[1]. Stability: 7 days at 2-8ºC.

MATERIAL REQUIRED BUT NOT PROVIDED
- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25ºC, 30ºC o 37ºC (± 0.1ºC).
- Matched cuvettes 1.0 cm. light path.

General laboratory equipment.

TEST PROCEDURE
1. **Assay Conditions**
- Wavelenght : ..... 340 nm.
- Cuvette: ..... 1 cm light path.
- Constant temperature .....25ºC / 30ºC / 37ºC.

2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette (note 1):

WR (mL.)	1.0
Sample (µL.)	100

4. Mix and incubate for 1 minute.
5. Read the absorbance (A) of the sample, start the stopwatch and read absorbance at 1 min interval thereafter for 3 min.
6. Calculate the difference of absorbance and the average absorbance difference per minute (ΔA/min.)

CALCULATIONS
GPT/ALT U/L. = ΔA/min. x 1750 (note 2)

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Temperature conversion factors
To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25ºC	30ºC	37ºC
25ºC	1.00	1.32	1.82
30ºC	0.76	1.00	1.39
37ºC	0.55	0.72	1.00

QUALITY CONTROL
Control sera are recommended to monitor the performance of the procedure, Control Normal Ref. QC001 and Control Pathological Ref. QC002. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions

REFERENCE VALUES[1]

	25ºC	30ºC	37ºC
Men up to	22 U/L	29 U/L	40 U/L
Women up to	18 U/L	22 U/L	32 U/L

Normal newborns have been reported to show a reference range of up to double the adult, attributed to the neonate's hepatocytes. These values decline to adult levels by approximately 3 months of age.
(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE
The ALT is a cellular enzyme, found in highest concentration in liver and kidney. High levels are observed in hepatic disease like hepatitis, diseases of muscles and traumatisms, its better application is in the diagnosis of the diseases of the liver.
When they are used in conjunction with ast aid in the diagnosis of infarcts in the myocardium, since the value of the alt stays within the normal limits in the presence of elevated levels of ast[1,4,5].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE
- *Measuring Range*:
  From detection limit of 3.9 U/L. to linearity limit of 260 U/L.
  If results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L. and multiply result by 10.

Precision:	Intra-assay n= 20		Inter-assay n= 20	
Mean (U/L)	34.9	118.4	34.1	118.3
SD	0.64	1.17	1.03	1.53
CV (%)	1.84	0.99	3.04	1.29

- *Sensitivity:* 1 U/L = 0.00052 ΔA/min
- *Accuracy:* Results obtained GPL (x) reagents did not show systematic differences when compared with other commercial reagents (y).
  The results obtained using 100 samples were the following:
  Correlation coefficient (r): 0.9946
  Regression Equation: y=1.0081x + 0.3629
  The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES
- Anticoagulants currently in use like heparin, EDTA, oxalate and fluoride do not affect the results. Haemolysis interferes with the assay[1]
- A list of drugs and other interfering substances with AST determination has been reported by Young et. al[2,3].

NOTES
1. Use clean disposable pipette tips for its dispensation.
2. Formulation to reach constant:

$$\Delta A/\text{min} \times 1750^* = \text{U/L of ALT}$$

$$^* \frac{Tv \times 1000}{\varepsilon \times LP \times Sv}$$

Tv= Total Volume in mL
ε NAD= 6.22 at 340 nm
LP= Light path
Sv= Simple volume in mL

BIBLIOGRAPHY

1. Murray R. Aspartate aminotransferase. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1112-116.
2. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
3. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
4. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
5. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

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