Reactivos GPL

Barcelona, España

CE

- LDH LQ -

LDH

Piruvato. Cinética. Líquida

Store at: +2+8ºC.

Presentation:
Cod. EZ021LQ CONT: R1 1 x 100 R2 1 x 25 mL .
EZ022LQ CONT: R1 2 x 100 R2 2 x 25 mL .

Procedure

Quantitative determination of lactate dehydrogenase (LDH).

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

TEST SUMMARY

Lactate dehydrogenase (LDH) catalyses the reduction of pyruvate by NADH, according the following reaction:

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-lactate} + NAD^+$$

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

REAGENTS COMPOSITION

R 1 Buffer	Phosphate pH 7.8 Pyruvate	80 mmol/L 0.6 mmol/L
R 2 Substrate	NADH	0.18 mmol/L

REAGENT PREPARATION AND STABILITY

Working reagent (WR):
Mix 4 volumes of R1 with 1 volumes of R1.
Stability: 15 days at 2-8ºC or 5 days at room temperature (15-25ºC).
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[1]. Separated from cells as rapidly as possible. Do not use oxalates as anticoagulants since they inhibit the enzyme.
Do not use haemolysed samples.
Stability: 2 days at 2-8ºC.

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

General laboratory equipment.

TEST PROCEDURE
1. Assay Conditions
- Wavelength : ..... 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:

	25ºC. – 30ºC	37ºC.
WR (mL)	3.0	3.0
Sample (µL.)	100	50

4. Mix. 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(Note 2)

25º- 30ºC ΔA/min x 4925* = U/L LDH
37ºC ΔA/min x 9690* = U/L LDH

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	1.33	1.92
30ºC	0.75	1.00	1.43
37ºC	0.52	0.70	1.00

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, Normal and Pathological.
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 if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

25ºC	30ºC	37ºC
120-240 U/L	160-320 U/L	230-460 U/L

(These values are for orientation purpose).
It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE

Lactate dehydrogenase (LDH) is an enzyme with wide tissue distribution in the body.
The higher concentrations of LDH are found in liver, heart, kidney, skeletal muscle and erythrocytes.
Increased levels of the enzyme are found in serum in liver disease, myocardial infarction, renal disease, muscular dystrophy and anemia[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 4 U/L to *linearity limit* of 1450 U/L, under the described assay conditions.
  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)	337	548	345	553
SD	4.63	5.11	5.27	7.68
CV	1.37	0.93	1.53	1.38

- Sensitivity: 1 U/L = 0.00029 ΔA/min
- Accuracy: Results obtained GPL reagents did not show systematic differences when compared with other commercial reagents.
  The results obtained using 50 samples were the following:
  Correlation coefficient (r): 0.9925
  Regression Equation: y=1.0059x - 1.1072

  The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES
- Haemolysis interferes with the assay.
- Some anticoagulants such as oxalates interfere with the reaction[1].
- A list of drugs and other interfering substances with LDH 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:.

ΔA/Min. x 4925* or 9690* = U/L LDH

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

Tv= Total volume in mL
ε NAHD = 6.22 at 340 nm
LP= Light path
Sv= Sample volume in mL

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