LDH

Pyruvate. Kinetic UV. DGKC

Quantitative determination of lactate dehydrogenase (LDH)
IVD

Store at 2-8ºC

PRINCIPLE OF THE METHOD

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

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

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

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.

REAGENTS

R 1 Buffer	Imidazol Pyruvate	65 mmol/L 0,6 mmol/L
R 2 Substrate	NADH	0,18 mmol/L

PRECAUTIONS

R1: H360- May damage fertility or the unborn child.
Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

Working reagent (WR):
Dissolve (→) 1 tablet of R2 in one vial of R1.
Cap and mix gently to dissolve contents.
Stability: 2 days at 2-8ºC or 12 hours at room temperature (15-25ºC).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.
Do not use the tablets if appears broken.
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.

ADDITIONAL EQUIPMENT

- 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.

SAMPLES

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.

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:

	25º - 30ºC	37ºC
WR (mL)	3,0	3,0
Sample (µL)	100	50

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

CALCULATIONS

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 assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
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; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 2 U/L to *linearity limit* of 1500 U/L.
If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L and multiply the result by 10.
Precision:

	Intra-assay (n= 20)		Inter-assay (n= 20)	
Mean (U/L)	388	731	402	757
SD	7,44	12,49	12,45	16,96
CV (%)	1,92	1,71	3,10	2,24

Sensitivity: 1 U/L = 0,00010 ΔA/min.
Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r)[2]: 0,987.
Regression equation: y= 1,6383x - 57,4835.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

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

SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. Pesce A. Lactate dehydrogenase. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1124-117, 438.
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.

PACKAGING

Ref: 1001260	Cont.	R1: 20 x 3 mL ,R2: 20 → 3 mL
Ref: 1001261		R1: 1 x 150 mL, R2: 10 → 15 mL

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