LDH

Pyruvate DGKC Method KINETIC

2x50ml

Intended Use:

Lactate Dehydrogenase (LDH) test reagent/kits is a medical device intended for the estimation of enzyme Lactate Dehydrogenase (LDH) and its isozymes in serum or plasma.

Clinical Significance

The enzyme activity found in circulation is a mixture of five isoenzymes. Each organ has a characteristic isoenzyme profile. Leakage of these isoenzymes from a diseased organ results in the elevation of total serum LD.

I*ncreased* levels become evident 8-12 hours after myocardial infarction reaching a maximum 4-5 days later. Elevated values in serum are also encountered in cases of pulmonary embolism and in about one third of patients with renal disease, specially those with pyelonephritis or tubular necrosis. In toxic hepatitis with jaundice, Hodking´s disease and abdominal and lung cancers elevations are specially high.

Moderate increases are also observed in cases of liver disease, megaloblastic and pernicious anemia and progressive muscular dystrophy.

Decreases are not important clinically.

Principle

Lactate dehydrogenase (LD/LDH) catalyzes the reduction of pyruvate to lactate (P-L) in the presence of reduced nicotinamide adenine dinucleotide (NADH) at pH 7.5.

The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD^+ proportional to the activity of LD present in the sample.

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow[\text{pH 7.5}]{\text{LD/LDH}} \text{L-Lactate} + NAD^+$$

This test has been formulated according the standarized method described by SFBC.

Reagents

Each LDH kit contains

Reagent 1 **LDH substrate**	TRIS buffer pH pyruvate sodium chloride	100 mmol/L 7.5, 2.75 mmol/L222 mmol/L.
Reagent 2 **LDH coenzyme**	NADH	1.55 mmol/L

Storage Instructions and Reagent 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 reagents over the expiration date.

Signs of reagent deterioration:

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

Reagent Preparation

Working reagent (WR):

Mix 4 vol. (R1) Substrate + 1 vol. (R2) coenzyme

Stability: 2 months at 2-8ºC Protected from light.

Materials required

- Photometer or spectrophotometer with a thermostatted cell compartment set at 30/37ºC, capable of reading at 340 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

Specimens

Serum free of hemolysis separated from the cells as soon as possible after collection. The use of heparin and citrate as anticoagulants have been reported to falsely elevate LD activity. Freezing results in loss of activity of the enzyme.

Assay 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

Reaction temperature	37ºC
WR (µL)	1000 µL
Sample (µL)	20µL

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

Application sheets for automated systems are available on request.

Calculation

U/L = ΔA/min x 8095

Samples with ΔA/min exceeding 0.150 at 340 nm should be diluted
1:10 with saline and assayed again. Multiply the results by 10.

If results are to be expressed as SI units
apply: U/L x 0.01667 = µkat/L

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 liter of sample (U/L).

Quality Control

Commercially available normal and pathological control sera are recommended to monitor the performance of the procedure.

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

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Reference Range[1]

30ºC	37ºC
140-280 U/L	207-414 U/L

These values are for orientation purpose; each laboratory should establish its own reference range.

Performance Characteristics

Measuring range:

From detection limit of 15.73 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:

U/L	Within-run		Between-run	
Mean	495	879	495	879
SD	4.32	7.34	14	13.5
CV%	0.87	0.83	2.83	1.54
N	10	10	10	10

Sensitivity: 1 U/L = 0.00029 ΔA/min.

Accuracy: Results obtained (y) did not show systematic differences when compared with other commercial reagents (x). The results obtained using 100 samples were the following:

Correlation coefficient (r): 0.9925.

Regression equation: y= 1.031x +3.147.

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

Interference:

- Lipemia (intralipid > 20 g/L) does not interfere.
- Bilirubin (> 40 mg/dL) does not interfere.
- Hemoglobin (> 4 g/L) may affect the results.
- Other drugs and substances may interfere[3].

Notes

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory

Literatures

1.Comission Enzymologie de la Societé Française d Biologie Clinique. Ann. Biol. Clin. 40: 123-128 (1982).

2. Sociedad Española de Química Clínica, Comité Científico, Comisión de Enzimas. Método recomendado para la determinación en rutina de la concentración catalítica de lactato deshidrogenada en suero sanguíneo humano. *Quim Clin* 1988; 8:57-61.

3. Young DS. Effects of drugs on clinical laboratory tests, 4th ed. AACC Press1995.

4. Tietz. N.W. Clinical Guide to Laboratory Tests, 3rd Edition. W.B. Saunders Co. Philadelphia, PA. (1995).

5. Friedman and Young. Effects of disease on clinical laboratory tests, 5th ed. AACC Press, 2000.

SYSTEM PARAMETERS

SYSTEM PARAMETERS		
Mode	:	Kinetic
Reaction	:	Descending
Wavelength	:	340 nm
Blank with	:	Distilled water/Air
Sample Volume	:	20 µL
Reagent Volume	:	1000 µL
Lag Time	:	60 (Sec)
Measuring Time	:	180 (Sec)
Interval time	:	60 (Sec)
No. of intervals	:	3
Factor	:	8095
Sensitivity	:	15.73 U/L
Linearity limit	:	Up to 1500 U/L

For *in vitro* Diagnostic use only.