LDH. Pyruvate. Kinetic UV. DGKC. Liquid	Ref.:LDH-011
Quantitative Determination of lactate dehydrogenase (LDH)	
Only for *in vitro* use in clinical laboratory	
Store at 2-8ºC	60 + 15 mL

LDH

PRINCIPLE OF THE METHOD

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].

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

Reagent 1 Buffer	Imidazol Pyruvate	65 mmol/L 0.6 mmol/L
Reagent 2 Substrate	NADH	0.18 mmol/L

PREPARATION

Working reagent (WR) :

Mix: 4 vol. (R1) Buffer + 1 vol. (R2) Substrate

Stability: 15 days at 2-8ºC or 5 days at 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 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 minute intervals thereafter for 3 minutes.
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.
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 4 U/L to linearity limit of 1450 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)	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 using BSM reagents (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= 1.0059x – 1.1072.
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].

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

BIBLIOGRAPHY

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