LDH-P LS 4+1

IVD

Quantitative determination of LDH in Serum or Plasma. Kinetic UV method. Pyruvate substrate. SFBC modified optimized.

REF CC1252 R1:3x20 mL + R2: 1x15 mL **REF CC1250** R1:3x40 mL + R2: 1x30 mL **REF CC1254** R1: 3x80 mL + R2: 1x60 mL

METHOD AND PRINCIPLE

The Lactic Dehydrogenase (LDH) catalyzes the transformation of Pyruvate into L-Lactate with consequent oxidation of NADH in NAD^+. This determines a decrease in Absorbance (Abs) of the reaction which is measured photometrically at the wavelength of 340 nm.
The rate of decrease of the Abs is proportional to the activity of the LDH enzyme in the sample under examination.

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+ + H_2O$$

CLINICAL SIGNIFICANCE

LDH is a tetramer formed by the combination of two different monomers, coded by two distinct genes: the type H (H from English heart), more present in the heart, and the type M (M from English muscle), characteristic of skeletal muscle. From the different monomeric composition, there are five isoenzymatic forms: H4 or LDH1, H3M1 or LDH2, H2M2 or LDH3, H1M3 or LDH4, M4 or LDH5 which differ in their structural composition, biochemical properties and tissue diffusion.

- LDH1 (H4) is prevalent in the myocardium and red blood cells. Also present in the renal cortex and skeletal muscle.
- LDH2 (H3M1) is prevalent in the myocardium and in the red blood cells, as well as being present in the pancreas, renal cortex, lung and skeletal muscle.
- LDH3 (H2M2) is present in the lungs, placenta, skeletal muscle and pancreas.
- LDH4 (H1M3) is found in the renal medulla, skeletal muscle, lung and placenta.
- LDH5 (M4) is characteristic of muscle and liver. Also present in the renal medulla and pancreas.

Increased values of LDH1 and LDH2 are found in myocardial infarction and in hemolytic anemia. In particular, the level of LDH1, following myocardial infarction, reaches its peak after 48 h and remains altered for 1-3 weeks. Increased LDH3 is related to pulmonary infarction while a greater amount of LDH5 is characteristic of acute viral hepatitis.
A higher total LDH level than normal is found in diseases such as: myocardial infarction, pulmonary infarction, acute viral hepatitis, toxic hepatitis, shock state, severe anemia, muscular dystrophy, polymyositis, dermatomyositis, intense muscle exercise, diabetes, renal failure, cirrhosis of the liver, Reye's syndrome, leukemia and neoplasia. Decreased values are found in subjects exposed to ionizing radiation.

REAGENT COMPOSITION

Reagent (R1)

TRIS, pH 7.50	100 mmol/L
Pyruvate	2.75 mmol/L

Reagent (R2)

NADH	0.18 mmol/L

REAGENT PREPARATION AND STABILITY

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light.
Do not freeze the reagents.
Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera or Absorbance of Working Reagent at 340 nm < 1.000 in 1 cm cuvette.
After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid contamination, degradation from direct light and evaporation.
Substrate Start:
The Reagents are ready-to-use.

Sample Start:
Mix 4 parts of R1 + 1 part of R2
(E.g. 20 mL R1 + 5 mL R2) = Working Reagent
Stability: 30 days at +2° to +8°C, 5 days at +15° to +25°C
The Working Reagent must be protected from light.

SPECIMEN

Serum, heparin plasma or EDTA plasma.
Loss of activity within 3 days: at 2° - 8 °C < 8 % , at 15° - 25 °C < 2 %.
Stability at -20 °C at least 3 months.
Discard contaminated specimens.

PROCEDURE

Wavelength:	340 nm
Temperature:	37°C
Measurement:	against distilled water

Sample Start procedure:

Working Reagent	1000 µL
Sample	25 µL

Mix, read Absorbance after 1 minute and start stopwatch. Read Absorbance again after 1, 2 and 3 minutes. Calculate ΔAbs/min (average).

Substrate Start procedure:

Reagent (R1)	800 µL
Sample	25 µL

Mix and add reagent (R2) after 1 minute:

Reagent (R2)	200 µL

Mix, read Absorbance after 1 minute and start stopwatch. Read Absorbance again after 1, 2 and 3 minutes. Calculate ΔAbs/min (average).

CALCULATION

Calculation Factor (reading at 340 nm in 1 cm cuvette):

LDH (U/L) = ΔAbs/min x 8199

Multiparametric Calibrator:
Calculate a specific factor using a certificate multiparametric calibrator:

$$\text{Factor} = \frac{\text{Calibrator Concentration}}{\Delta\text{Abs/min (average).}}$$

LDH (U/L) = ΔAbs/min x Factor

Conversion Factor: LDH [U/L] x 0.0167 = LDH [µkat/L]

CALIBRATION

The results depend on the accuracy of the calibration, on the correct setting of the test on the instrument, on the correct volumetric ratio of reagent / sample and on the correct analysis temperature.
Use **MTD DiagnosticsChemistry Multicalibrator**:

Chemistry Multicalibrator - REF CAL1010 (10x3 mL)

QUALITY CONTROL

Normal and abnormal control sera of known enzymatic activity should be analysed routinely with each group of unknown samples utilizing **MTD Diagnostics** Quality Control Material:

Chemistry Control N - REF CNN1010 10 x 5 mL (Level 1)
Chemistry Control P - REF CNP1020 10 x 5 mL (Level 2)

EXPECTED VALUES

Adults: < 480 U/L

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

PERFORMANCE

PRECISION:
Low Level: Samples= 20; Average = 495; S.D. = 4.32; CV = 0.87%
High Level: Samples = 20; Average = 879; S.D. = 7.34; CV = 0.83%

ACCURACY (CORRELATION): A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

y=1.00x + 1.646 ; r=0.999

SENSITIVITY: 20 U/L

LINEARITY: 20 - 650 U/L

SPECIFICITY / INTERFERENCES

No interference was observed by Bilirubin up to 20 mg/dL and Lipemia up to 1,000 mg/dL Triglycerides. Haemoglobin interferes because LDH is released by erythrocytes.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact your 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 data.

PRECAUTIONS

The product does not contain any other hazardous substances or mixtures according to EC Regulation No. 1272/2008 (CLP) or their concentrations are such that they are not considered to be persistent, bioaccumulative or toxic (PBT). Therefore, it is not subject to the special labeling required by the aforementioned regulation. The product is labeled according to the directive for CE marking (98/79 / EC). Sodium Azide less than 0.1%.
However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:
S26 (P305 - P351 - P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 (P302 - P352): After contact with skin, wash immediately with plenty of water.
S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.
S46 (P301 - P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.
S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.
S63 (P304 - P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.
All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.
FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.

SIMBOLOGY

CE	CE Mark (EC Directive 98/79)		
IVD	In Vitro Diagnostic		Temperature Limitation
	Consult instructions for use		Contains sufficient for <n> test
REF	Catalog Number		Use By
LOT	Batch Code		Manufacturer

BIBLIOGRAPHY

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