LDH-P – Instructions for Use (IFU)

ISE S.r.l.
CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

R1: 4 x 50 mL – R2: 4 x 20 mL • REF A-R0200001201

R1: 3 x 18,5 mL – R2: 1 x 18,5 mL • REF R3330000021

IVD CE

INTENDED USE
Product for use in the quantitative determination in vitro of the LDH activity in human serum. The results of the test must always be interpreted in conjunction with the clinical context. For professional use only.

CLINICAL SIGNIFICANCE
LDH is an enzyme which catalyzes the reduction of pyruvic acid to lactic acid. The wide-spread diffusion of LDH in the organism explains why this enzyme increases in the serum in numerous pathological conditions involving various tissues, such as the liver (viral hepatitis), cardiac muscle (myocardial infarction), skeletal muscle and kidneys. The kidney is the organ containing the highest concentration of the enzyme, followed by the myocardium, skeletal muscle, spleen, liver and lungs. LDH activity in the serum is determined by a group of five isoenzymes deriving from different tissues. LDH activity can be determined by measuring the conversion of lactate to pyruvate or pyruvate to lactate. Hill and Levi in 1954, Wròblewski and La Duein 1956, and Blachaer in 1961 developed methods based on the use of NADH. The lmethod has been optimized according to the suggestions proposed by the Scandinavian Society for Clinical Chemistry (1).

PRINCIPLE
LDH catalyzes the following reaction:

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{lactate} + \text{NAD}^+$$

The rate of NADH oxidation is proportional to the LDH activity.

Abbreviations:
LDH: Lactic dehydrogenase (EC 1.1.1.27)
NADH: Reduced Nicotinamide-adenine dinucleotide
NAD+: Oxidated Nicotinamide-adenine dinucleotide

REAGENTS
A-R0200001201 – R1: 4 x 50 mL – R2: 4 x 20 mL
Reagente 1: n° 4 vials x 48,0 mL ready for use
Reagente 2: n° 4 vials x 6,0 mL ready for use
R3330000021 – R1: 3 x 18,5 mL – R2: 1 x 18,5 mL
Reagente 1: n° 3 vials x 12,6 mL ready for use
Reagente 2: n° 1 vial x 4,4 mL ready for use

Concentrations

Reagent 1: Substrate buffer		
	Conc.	**U.M.**
TRIS buffer pH 7.4 ± 0.2	50.1	mM
Sodium pyruvate	1.20	mM
EDTA	5.00	mM
Sodium azide	13.8	mM
Reagent 2: NADH		
	Conc.	**U.M.**
TRIS buffer pH 10.2 ± 0.2	50.0	mM
NADH (from yeast)	1.80	mM
Sodium azide	13.8	mM

Precautions
Kit for professional laboratory use, used only by qualified and properly trained technical personnel, under the supervision of a doctor in charge of the laboratory. In addition to any risk statements relating to active components, reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in EU Regularion1272/2008 EC and subsequent amendments and additions. However, it is recommended to handle the reagents according to the rules of good laboratory practice.

Reports of serious incidents
In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements. Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

SAMPLE COLLECTION
Type of sample and storage
Use fresh non-haemolysed serum samples.
LDH is stable in serum 1 week at 4 - 20°C and one month at - 20°C (2).

Precaution
All human samples must be handled and disposed of as potentially infectious materials.

PROCEDURE
Quality control
Control sera with known levels of LDH-P are commercially available for quality control, including certificates of analysis showing values and confidence limits. Normal and pathological control sera are available as "Normal control serum" cod. R0400000006 and "Pathological control serum" code R0400000106. The values obtained must be contained within the acceptability range. In case of incorrect results check the following points:

- Cleanliness of glassware.
- Wavelength setting.
- Expiration date of reagents.

Automation
Although this device has been developed and manufactured to be used by manual method and instrumental systems, it can also be used in combination with other instrumental devices that are able to meet the requirements indicated in the paragraph "Reaction conditions / Technique".
All applications that are not explicitly approved cannot be guaranteed in terms of performance and will therefore have to be evaluated by the user

Calibration
For calibration use the “Multicalibrator” code R0300000006. For the ISE srl instruments, the calibration is recommended every 10 days.

Traceability
The LDH value is reported in the package insert supplied with the “Multicalibrator”.

Calibration Stability
Reaction conditions

Wavelength (primary):	340 nm
Wavelength (secondary):	380 nm
Temperature:	37°C

Technique – Procedure with reagent B as starter
Bring the reagents to the reaction temperatures.

	U.M.	**Calib.Serum**	**Sample**	**Biank**
Reagent	µL	900	900	900
Sample	µL	-	20	-
Water	µL	-	-	20
Mix well and incubate at 37° for 3 min. and add				
Reagent B	µL	100	100	100

Mix well, take the reading at 37°C.
Read the absorbances every minute of the sample and of the calibration serum by subtracting the absorbance of the reagent blank for 5 minutes, determine the delta/minute of reaction.
The reaction volumes can be varied proportionally, the calculation remaining unchanged.

Results
The concentration of Lactic Dehydrogenase is obtained through the following formula:

$$\frac{\Delta \text{ D.O. Sample}}{\Delta \text{ D.O. Calib.Serum}} \; x \; \text{Conc. Calib.Serum (U/L)} = \text{U/L LDH}$$

Calculation of results obtained against multiplication factor

Δ D.O./min x K-factor* = U/L LDH

Explanation of the formula:

$$\frac{\text{Vt x 1000}}{\text{M.E.C. x O.P. x Vc}} = \text{K – factor} * \text{ x } \Delta \text{ D.O./min.} = \text{U/L LDH}$$

*K-factor = 8199
where:
U/L = activity in international units per litre
Δ D.O./min. = change in absorbance per minute
Vt = total reaction volume (µL)

Sclavo Diagnostics International
Via Po 26-28 – Loc. Pian dei Mori – 53018 Sovicille (SI) (Italy)
Phone +39 0577 39041 - Fax +39 0577 390 444

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1000 = conversion of concentration per litre
C.M.E. = coeffic. NADH extinction micromolar 6.22 cm2/µmol at 340 nm
P.O. = optical path (1,0 cm)
Vc = sample volume in the final reaction mixture (µL)

Materials included in the kit
The reagents are described above.

Materials required but not supplied in the kit
Calibrators and controls.

NORMAL VALUES
Serum: 135 - 460 U/L
Each laboratory should calculate its own normal values based on its local population.

ANALYTICAL CHARACTERISTICS / PERFORMANCE
Linearity
If at 340 nm is higher than 1600 U/L repeat the test, diluting the serum 1:10 in physiological saline. Multiply the result by 10.

Specificity
This method is specific for LDH. The substrate concentration is optimized for the isoenzymes normally present in the serum. Any nonspecific reaction is terminated during the lag time before taking the reading.

Accuracy-Recovery
The recovery of pure LDH added to normal sample at known titer was 100.2%.

Precision of the method

Within-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	236.87	6.711	2.83%	20
High	U/L	839.55	22.426	2.67%	20
Between-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	236.87	6.607	2.79%	20
High	U/L	839.55	43.892	5.23%	20

Sensitivity
At λ 340 nm, a concentration of about 28.5 U/L of Lactic Dehydrogenase in the conditions established for this test.

Comparative method
The LDH-P was compared with the method described by SCE (1). The following results were found: Linear regression Y = 0.9782x -14.56;
Correlation coefficient: r = 0.995; Samples tested = no. 71.

Disposal of reagent
Disposal of reagents must be performed in accordance with the EC regulations regarding waste or the local national or regional legislation.

Manufacturer:
Sclavo Diagnostics International
Via Po 26-28 – Loc. Pian dei Mori – 53018 Sovicille (SI) (Italy)
Phone +39 0577 39041 - Fax +39 0577 390 444

Distributor:
I.S.E S.r.l.
Via Delle Driadi, 45 – 00133 Roma
Tel.+39 077 4579365; FAX +39 077 4579305
E-mail: info@logotech-ise.com
www.logotech-ise.com

Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

References

1. The Committee on Enzymes of the Scandinavian Society for Clinical Investigation and Clinical Physiology. Scand J Clin Lab Invest 1974; 32: 291
2. Amador EG, Wacker WE. Methods Biochem Anal 1965; 13: 265-356

REVISION	DATE	CHANGE
Rev.A	12/2022	New Issue for IVDR Regulation (UE) 2017/746 compliance