ISE S.r.l. **LDH-P** – Instructions for use

CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

CE IVD

R1: 2x 18 mL R2: 2x4,5 mL **R3330000021** REF

Diagnostic reagent for quantitative in vitro determination of lactate dehydrogenase (LDH) in human serum or plasma on photometric systems

TEST PARAMETERS

Method:	UV, kinetic, decreasing reaction, optimized DGKC
Wavelength:	340 nm, Hg 334 nm, Hg 365 nm
Temperature:	25 °C, 30 °C, 37 °C
Sample:	Serum, heparin or EDTA plasma
Linearity:	up to 1200 U/L on automated systems
Sensitivity:	The lower limit of detection is 5 U/L

SUMMARY [1,2]

Lactate dehydrogenase (LDH) is an enzyme, consisting of five different isoenzymes which catalyse the interconversion of L-lactate and pyruvate. LDH is present in the cytoplasm of all human tissues with higher concentrations in liver, heart and skeletal muscle, and lower values in erythrocytes, pancreas, kidney and stomach. Increased LDH activities are found in a variety of pathological conditions such as myocardial infarction, cancer, diseases of liver, blood or muscle. However, because of the lack of organ specificity, determination of its isoenzymes or other enzymes such as alkaline phosphatase or GPT (ALT) / GOT (AST) is necessary for differential diagnosis.

TEST PRINCIPLE

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

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1:	
Phosphate buffer, pH 7.5	64 mmol/L
Pyruvate	0.80 mmol/L
Reagent 2:	
Good´s buffer, pH 9.6	
NADH	1.0 mmol/L

REAGENT PREPARATION

Substrate Start:

The reagents are ready to use.

Sample Start:

Mix 4 parts of Reagent 1 with 1 part of Reagent 2 (= working reagent).

REAGENT STABILITY AND STORAGE

Conditions: Protect from light (R2)

Close immediately after use

Avoid contamination

Do not freeze the reagents.

Substrate Start:

Storage: at 2 – 8 °C

Stability: up to the indicated expiration date

Sample Start (working reagent):

Stability:	at 15 – 25 °C	8 hours
	at 2 – 8 °C	5 days

The working reagent must be protected from light!

SAMPLE STABILITY AND STORAGE [4]

Serum, heparin plasma or EDTA plasma

Stability:	at 20 – 25 °C	4 days
	at 2 – 8 °C	6 weeks

Discard contaminated specimens.

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)

General laboratory equipment

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Substrate Start

Pipette into test tubes	25 °C, 30 °C	37 °C
Reagent 1	1000 µL	1000 µL
Sample	20 µL	10 µL
Mix. Incubate for approximately 1- 5 min. Then add:		
Reagent 2	250 µL	250 µL
Mix. Read initial absorbance against air after 1 minute and start a timer. Read absorbance again after exactly 1, 2 and 3 min. Determine ΔA/min. during the linear part of the assay.		

Sample Start

Pipette into test tubes	25 °C, 30 °C	37 °C
Working reagent	1000 µL	1000 µL
Sample	20 µL	10 µL
Mix. Read initial absorbance against air after 1 minute and start a timer. Read absorbance again after exactly 1, 2 and 3 min. Determine ΔA/min. during the linear part of the assay.		

CALCULATION

With factor: (light path 1 cm)

LDH [U/L] = ΔA/min x Factor

Factors:

Substrate Start

	25 °C or 30 °C	37 °C
Factor at 340 nm	10080	20000
Factor at 334 nm	10275	20390
Factor at 365 nm	18675	37060

Sample Start

	25 °C or 30 °C	37 °C
Factor at 340 nm	8095	16030
Factor at 334 nm	8250	16345
Factor at 365 nm	15000	29705

With calibrator:

$$LDH\ [U/L] = \frac{\Delta A/min\ Sample}{\Delta A/min\ Calibrator} \times \text{activity calibrator [U/L]}$$

UNIT CONVERSION

U/L x 0.01667 = µkatal/L

REFERENCE RANGE [6] *

	25°C	30°C	37°C	Unit
Adults	< 240	< 346	< 480	[U/L]
	< 4	< 5.77	< 8	[µkat/L]

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

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PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

On automated systems the test is suitable for the determination of LDH activities up to 1200 U/L.
In case of a manual procedure, the test is suitable for LDH activities, which correspond to a maximal ΔA/min of 0.15 at 340 and 334 nm or 0.08 at 365 nm.
If these values are exceeded, the sample should be diluted 1 + 10 with NaCl solution (9 g/L) and results multiplied by 11.

SENSITIVITY / LIMIT OF DETECTION

The lower limit of detection is 5 U/L

PERFORMANCE CHARACTERISTICS

Intra-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	142	5.50	3.86
Sample 2	245	4.95	2.01
Sample 3	497	8.39	1.69

Inter-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	144	3.09	2.13
Sample 2	248	4.53	1.82
Sample 3	492	6.23	1.26

SPECIFICITY / INTERFERENCES

No interference up to:

Ascorbic acid	30 mg/dL
Bilirubin	40 mg/dL
Triglycerides	2000 mg/dL

Hemolysis interferes because LDH is released by erythrocytes.

For further information on interfering substances refer to Young DS [5].

METHOD COMPARISON

A comparison of our LDH-P (y) with a commercially available test (x) using 78 samples gave following results:

y = 1.03 x + 2.13 U/L; r= 0.999.

CALIBRATION

The use of a LDH Calibrator is optional.
We recommend our multi calibration serum. This method is traceable to the molar extinction coefficient.

QUALITY CONTROL

All control sera with LDH values determined by this method can be used.
We recommend the our serum controls (control serum with values in the normal and abnormal range).
Each laboratory should establish corrective action in case of deviations in control recovery.

AUTOMATION

Special adaptations for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. In very rare cases, samples of patients with gammopathy might give false results [7].
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
4. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

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Symbols on labels and packaging

IVD = In vitro diagnostic medical device
REF = Catalog Number
LOT = Lot Number
= Manufacturer
= Expiration date
= Temperature limitation
= Instruction for use

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