

LDH liquid

Kinetic UV method

R1: 2 x 40 ml + R2: 2 x 10 ml

CL42-100

INTENDED USE

Kit for quantitative determination of Lactate Dehydrogenase enzyme, LDH (EC 1.1.1.27.), in serum and plasma.

CLINICAL MEANING

The enzyme LDH is present in all cytoplasmic cells. Higher LDH values lead back to diseases of various nature, for instance: hepatic (hepatitis), cardiac (myocardial infarction, congestive heart failure), muscular (traumas, myopathy), hematological, renal or pulmonary diseases; neoplasia.

PRINCIPLE

In presence of NADH, LDH transforms pyruvate in lactate and NAD⁺. NADH oxidation in unit time, measured at 340 nm, is proportional to the LDH concentration in the sample.

SAMPLE

Serum, EDTA or heparinized plasma. Do not use oxalate as anticoagulant. Avoid hemolyzed samples.

The LDH enzyme activity in serum decreases 10% after 5 days at 4-25°C.

REAGENTS

Only for in Vitro diagnostics. Liquid reagents ready to use. Reagents marked with an asterisk are considered dangerous.

Package content	CL42-100
REAGENT 1 Tris buffer (pH 7,5) 88 mmol/L, pyruvate 2.75 mmo/L, sodium chloride 200 mmol/L, sodium azide 15 mmol/L	2 x 40 ml
REAGENT 2 Tris buffer (pH 10.2) 10 mmol/L, NADH 2,4 mmol/L, sodium azide 15 mmol/L	2 x 10 ml

STABILITY: reagents are ready to use. Store at 2-8°C and protect from light to keep the reagents stable up to the expiration date on the label. Do not freeze. Once opened the reagents are stable for 2 months at 2-8°C if contamination is avoided. Keep bottles closed when not in use. Do not use turbid reagents.

NECESSARY ITEMS – NOT PROVIDED

Usual laboratory equipment: UV/VIS Spectrophotometer with temperature control; automatic micropipettes; Optical glass cuvettes or, alternatively, disposable ones in optical polystyrene; Saline solution.

PREPARATION OF WORKING REAGENT (only for monoreagent procedure)

Mix 10 volumes of Reagent 1 with 1 volume of Reagent 2.

Stability: 5 days at 20-25°C, 4 weeks at 2-8°C if stored in a closed bottle protected from light.

MANUAL ASSAY PROCEDURE

Analysis: decreasing kinetic
Wavelength: 340 nm (334 - 365)
Optical path: 1 cm
Temperature: 30 or 37°C
Rate Time: 3 minutes
Reading: against air or distilled water
Sample/Reagent 1/Reagent 2: 1/40/10

Bireagent procedure

Let the reagents reach the chosen temperature before the analysis.

Pipette in cuvette:

Sample	20 µl
Reagent 1	800 µl

Mix and incubate for 1 minute at 37°C. Add:

Reagent 2	200 µl
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Mix and pour into the test cuvette. Incubate at the test temperature for 1 minute. Read initial absorbance, repeat the reading at constant intervals of 1 minute for 3 minutes. Calculate the average value of the absorbance variations per minute ($\Delta A/\text{min}$).

Monoreagent procedure

Bring the working reagent to the chosen temperature before the analysis.

Pipette in cuvette:

Sample	20 µl
Working reagent	1,0 ml

Mix and pour into the test cuvette. Incubate at the test temperature for 1 minute. Read initial absorbance, repeat the reading at constant intervals of 1 minute for 3 minutes. Calculate the average value of the absorbance variations per minute ($\Delta A/\text{min}$).

Reaction volumes can be proportionally varied without any change in calculation.

CALCULATION

Calculate the enzymatic activity in the sample using the following formula:

Activity in U/l: $\Delta A/\text{min} \times 8252$

Activity in $\mu\text{kat/l}$: $U/l \times 0.0167$

REFERENCE VALUES

30°C	37°C
140 ÷ 275 U/L	200 ÷ 430 U/L

Each laboratory should define its own reference values for this method.

QUALITY CONTROL – CALIBRATION

All Clinical Chemistry laboratories should implement a quality control program. Control serums of human origin are available for this purpose on request:

PRE-NORM serums with normal values

PRE-PATH serums with pathological values

If the method requires it, a multiparameter calibrator of human origin is available.

PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of the method is 15 U/L.

Linearity

Up to 1500 U/L.

For higher values, dilute the samples 1:10 with saline solution and multiply the result by 10.

Precision

Within run (n=10)	Average (mmol/L)	SD (mmol/L)	CV %
Sample 1	302	3,2	1,1
Sample 2	486	7,6	1,6

Between run (n=20)	Average (mmol/L)	SD (mmol/L)	CV %
Sample 1	311	16	5,15
Sample 2	522	24	4,6

Interferences

Lipids up to 2000 mg/dl as triglycerides do not interfere. Up to 40 mg/dl of bilirubin does not interfere. Hemolysis presence in the sample gives falsely positive results.

Correlation against a reference method

The correlation of the method (Y) against a reference method (X) gives this equation:

$$Y = 1,0803X - 5,3694 \quad r = 0,9987$$

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

The reagents may contain non-reactive components and various preservatives. Contact with the skin and ingestion should be avoided. Use the normal precautions expected with correct behavior in laboratory.

REFERENCES

- Ann. Biol. Clin., 40, (1982), 123.
- Kaplan LA, Pesce AJ: "Clinical Chemistry", Mosby Ed. 1989

MANUFACTURER

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KEY SYMBOLS

	dispositivo medico diagnostico in vitro
	numero di lotto
	numero di catalogo
	limite di temperatura
	usare entro la data
	attenzione
	consultare le istruzioni d'uso

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