LDH Liquid

Kinetic method UV

R1: 5 x 50 ml + R2: 1 x 25 ml CL41-275

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	**CL41-275**
REAGENT 1 Tris buffer (pH 7,5) 88 mmol/L, pyruvate 1,6 mmo/L, sodium chloride 200 mmol/L, sodium azide 15 mmol/L	5 x 50 ml
REAGENT 2 Tris buffer (pH 10.2) 10 mmol/L, NADH 2,4 mmol/L, sodium azide 15 mmol/L	1 x 25 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:	1/50/5

Bireagent procedure

Let the reagents reach the chosen temperature before the analysis.

Pipette in cuvette:

Sample	20 µl
Reagent 1	1,0 ml

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

Reagent 2	100 µl

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 (ΔA/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 (ΔA/min).

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

CALCULATION

Calculate the enzymatic activity in the sample multiplying ΔA/min by the proper factor from the following table.

λ	Monoreagent procedure	Bireagent procedure
334 nm :	8065	8888
340 nm :	8252	9061
365 nm :	15000	16000

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 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

1 Ann. Biol. Clin., 40, (1982), 123.)
2 Kaplan LA, Pesce AJ: “Clinical Chemistry”, Mosby Ed. 1989

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalog number
	temperature limits
	use by
	caution
	consult accompanying documents

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