## **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	5 x 50 ml
chloride 200 mmol/L, sodium azide 15 mmol/L	
REAGENT 2	
Tris buffer (pH 10.2) 10 mmol/L, NADH 2,4 mmol/L, sodium	1 x 25 ml
azide 15 mmol/L	

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 μΙ
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  $(\Delta A/min)$ .

## Monoreagent procedure

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

Pipette in cuvette:

Sample	20 μΙ
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  $(\Delta A/min)$ .

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

### **CALCULATION**

Calculate the enzymatic activity in the sample multiplying  $\Delta A/min$  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

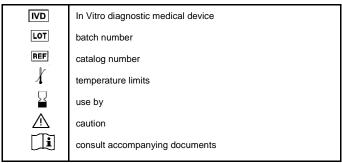
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## **KEY SYMBOLS**



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