Lactate dehydrogenase (LDH)-Liquizyme (1+1) E.C.1.1.1.27.

REF: 279 001 (2 x 25 ml)   50 test
REF: 279 002 (4 x 25 ml)   100 test

Intended Use

Spectrum Diagnostics liquizyme LDH reagent is intended for the in-vitro quantitative, diagnostic determination of LDH in human serum on both automated and manual systems.

Background

The lactate dehydrogenase (LDH) enzyme is widely distributed in heart, liver, muscle, and kidney. LDH catalyzes the conversion of lactate to pyruvate. The enzyme is a tetrameric protein and gives rise to five isoenzymes. Heart, kidney, brain and erythrocytes have the highest proportion of LD-1 and LD-2. Liver and skeletal muscle have highest percentage of LD-5. LDH is significantly increased during myocardial infarction. A maximum value is reached 48 hours after the onset of manifestation and persists up to 10 days .Elevated serum levels of LDH have also been observed in patients with megaloblastic anemia, disseminated carcinoma, leukemia and trauma. Mild increases in LDH activity has been reported in cases of haemolytic anemia, muscular dystrophy, pulmonary infarction, hepatitis, nepherotic syndrome and cirrhosis.

Method

Kinetic ultraviolet method.

Assay Principle

LDH catalyzes the reaction between pyruvate and NADH to produce NAD and L-Lactate:

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

The initial rate of the NADH oxidation is directly proportional to the catalytic LDH activity. It is determined by measuring the decrease in absorbance at 340 nm.

Reagents

Reagent 1 (R1 Buffer)
Phosphate buffer (pH 7.5)	50	mmol/L
Pyruvate	3.0	mmol/L
Sodium Azide	8.0	mmol/L

Reagent 2 (R2 Enzyme)
NADH	> 0.06	mmol/L
Sodium azide	8.0	mmol/L

For further information, refer to the Lactate dehydrogenase reagent material safety data sheet.

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Both reagents (R1) and (R2) contain sodium azide which may react with copper or lead plumbing.

Reagent Preparation, Storage and Stability

All reagents are stable until expiration date stated on label when stored refrigerated at 2 - 8 °C.Once opened,the reagent is stable for 2 months when stored at the specified temperature.

Working solution can be prepared by adding equal volumes from R1 and R2 .
Stability: 3 weeks at 2 – 8 °C or 2 days at 15 – 25 °C.

Deterioration

Do not use liquizyme LDH reagent if it is turbid or if the absorbance of the working reagent is less than 1.0 at 340 nm. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation

Use nonhemolyzed serum. Heparin is the only acceptable anticoagulant. Sodium citrate and EDTA have an inhibitor effect and must not be used.The biological half-life of LDH in serum is 10 - 54 hours.
Stability: 6 weeks at 4 – 8 °C ; 4 days at 20 – 25 °C
Freezing of the samples is not recommended.

System Parameters

Wavelength	340 nm (334 – 365 nm)
Optical path	1 cm
Assay type	Kinetic
Direction	decrease
Sample : Reagent Ratio	1 : 50
e.g.: Reagent volume	1 ml
Sample volume	20 ml
Temperature	37 °C
Equilibration time	30 seconds.
Read time	1 to 3 minutes
Zero adjustment	Against air
Reagent Blank Limits	Low 1.00 AU
	High 2.5 AU
Sensitivity	10 U/L
Linearity	1200 U/L

Procedure

Pipette into cuvette (37 °C)	
Working solution	1 ml (or add 0.5 ml R1 + 0.5 ml R2)
Specimen	20 μl

Mix, read initial absorbance after 30 secnods and start timer simultaneously. Read again after 1, 2 and 3 minutes. Determine the mean absorbance change per minute (∆A/min).

Calculation

To calculate the LDH activity use the following formula
$$U/L = 8095 \times \Delta A\ 340\ nm/min.$$

Quality Control

Normal and abnormal control serum of known concentrations should be analyzed with each run.

Performance Characterstics

Precision
Within run (Repeatiblity)

	Level 1	Level 2
n	20	20
Mean (U/L)	433	923
SD	6.8	6.64
CV%	1.57	0.71

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (U/L)	439	935
SD	7.1	6.71
CV%	1.62	0.79

Methods Comparison

A comparison between Spectrum Diagnostics LDH reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.967 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of this assay is 10 U/L.

Linearity

The reaction is linear up to LDH concentration of 1200 U/L; specimens showing higher concentration should be diluted 1+5 with physiological saline and repeat the assay (result×6).

Interfering substances

Haemolysis
Erythrocyte contamination elevates results significantly since LDH activities in erythrocytes are 150 times higher than those in normal sera.

Icterus
No significant interference.

Lipemia
Lipemic specimens may cause high absorbance flagging.Diluted sample may be recommended.

Anticoagulants
EDTA and citrate may inhibit the reaction.

Expected value (at 37 °C)

Adults   240 - 480 U/L   (4.0 - 8.0 mkat/L)

Children (7 - 12 Years)
Female   :   < 580 U/L   (< 9.65 mkat/L)
Male   :   < 764 U/L   (< 12.7 mkat/L)
Premature :   < 1103 U/L   (< 18.4 mkat/L)

Calculate for temperature conversion factor of
0.5 (37 ⟶ 25°C) and 0.67 (37 ⟶ 30°C).

Spectrum]iagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Analytical Range:

10 - 1200 U/L.

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

Reference

1. Dito WR. Lactate dehydrogenase :A brief review. In : Griffiths JC, ed. Clinical Enzymology. New York :masson publishing USA; 1979:18.
2. Kachmar JF , Moss DW: Enzymes. In Fundamentals of clinical chemistry. NW Tietz, editor, saunders, philadelphia, 1976 pp 652-6603.
3. Van der heiden C, B AIS, Gerh Ardt W,Rosallsis. Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 8. IFCC method for LDH.Eur J Clinical Chem Clin Biochem . 1994;32:639-655.
4. Young DS. Effects of drugs on clinical laboratory tests. AACC press, Washington D.C., 1990.
5. Zimmerman HJ, Henery JB : Clinical enzymology. In Clinical diagnosis and management by laboratory methods, 16 th ed., JB henery, editor, saunders, philadeLphia,1979, pp 365-368.

ORDERING INFORMATION

CATALOG NO.	QUANTITY
279 001	2 x 25 ml
279 002	4 x 25 ml