Spectrum For Diagnostic Industries

Lactate dehydrogenase (LDH)UV Kinetic(1+1)

IVD

REF.	Pack size
186 04 025	(4 x 25 ml) 100 tests

Intended Use

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

Introduction

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.

Principle

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

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

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

Buffer Reagent

Phosphate buffer (pH 7.5)	50 mmol/L
Pyruvate	3.0 mmol/L
Sodium Azide	8.0 mmol/L

Co-enzyme

NADH	> 0.18 mmol/L
Sodium azide	8.0 mmol/L

Reagents preparation, storage and stability

Prepare working solution by adding equal volumes from R1 and R2,Working solution is stable for 3 weeks at 2 – 8 ^{o}C or 2 days at 15 -25 ^{o}C.

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

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.

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.

Specimen collection and preservation

Use non-haemolyzed 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^{o}C ; 4 days at 20 – 25^{o}C
Freezing of the samples is not recommended.

Procedure

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 µl
Temperature	37 ^{o}C
Equilibration time	30 seconds
Read time	1 to 3 minutes
Zero adjustment	Against Dist. water
Reagent Blank Limits	Low 1.0 AU
	High 2.5 AU

Working solution 1 ml (or add 0.5 ml R1+ 0.5 ml R2)

Specimen 20 µl

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

Calculation

To calculate the LDH activity use the following formulae:
U/L = 8095 x ΔA 340 nm/min.

Quality control

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

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).

Interference

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 values

1- Adults : 240-480 U/L (4.0- 8.0 µkat/L)

2- Children (7-12 Years)
a) Female : < 580 U/L (< 9.65 µkat/L)
b) Male : < 764 U/L (<12.7 µkat/L)
c) Premature : < 1103 U/L (<18.4 µkat/L)

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

Performance characteristics

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

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

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.

References

1.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.
2. Zimmerman HJ, henery JB: Clinical enzymology. In: Clinical diagnosis and management by laboratory methods, 16 th., JB Henery, editor, saunders, philadelphia,1979.
3.Kachmar JF, Moss DW: Enzymes. In Fundamentals of clinical chemistry. NW Tietz, editor, saunders, philadelphia, 1976 pp 652-6603.

SYMBOLS IN PRODUCT LABELLING

IVD For in-vitro diagnostic use
LOT Batch Code/Lot number
REF Catalogue Number
Consult instructions for use
Temperature Limitation
Use by/Expiration Date
CAUTION. Consult instructions for use
Manufactured by

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