ARENA BioScien

Block 5, Street 9 inside The Ismailia free zone. Ismailia- Egypt
Post Code-41511
Tel.-Fax: +202 21813500/ +202 21813600
Mob.: +2 01211550941/ +2 01119225860
E-mail: sales@arenabioscientific.com
Website: www.arenabioscien.com

Lactate Dehydrogenase(LDH) (4+1)

Diagnostic reagent for the in-vitro quantitative determination of LDH in human serum and plasma on both automated and manual system.

REF: BS.1/LD05.005.0025	25 tests	REF: BS.1/LD10.005.0050	50 tests
REF: BS.1/LD05.010.0050	50 tests	REF: BS.1/LD02.025.0050	50 tests
REF: BS.1/LD04.025.0100	100 tests		

CLINICAL SIGNIFICANCE

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, nephrotic syndrome, and cirrhosis.

METHOD PRINCIPLE

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

Pyruvate + NADH + H+ $\xrightarrow{\text{LDH}}$ 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.

REAGENT COMPOSITION

Reagents:	Composition
R1: Buffer	
- Phosphate buffer (pH 7.5)	- 50 mmol/L
- Pyruvate	- 3.0 mmol/L
- Sodium Azide	- 8.0 mmol/L
R2: Starter	
- NADH	- >0.18 mmol/L
- Sodium Azide	- 8.0 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- 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.
- Respect country requirement for 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.

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

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien LDH reagent working solution is prepared as by mixing 4 volumes of buffer (R1) and 1volume of starter (R2), e.g. 400 µl R1 + 100 µl R2. After reconstitution working reagent is stable for 2 months at 2 – 8 ºC or 1 week at 15 - 25 ºC. All reagents are stable until expiration date stated on label when properly stored in an upright position and refrigerated at 2-8°C (do not freeze).

Deterioration
The ***BioScien*** LDH reagent considered damaged if it is turbid, do not use LDH reagent if it is turbid or if the absorbance of the working reagent is less than 1 at 340 nm. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

SPECIMEN COLLECTION AND PRESERVATION

Serum and Plasma
Nonhaemolyzed fresh serum is the preferred specimen. Heparin is the only acceptable anticoagulant. Complexing anticoagulants such as citrate, oxalate, and EDTA must be avoided.
The biological half-life of LDH in serum is 10 - 54 hours.
Stability: 6 months at 4 – 8 ˚C;
4 days at 20 – 25 ˚C

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 µl
Temperature	37 ˚C
Equilibration time	30 sec.
Zero adjustment	Against Air
Reagent Blank Limits	Low 1.0 AU High 2.5 AU
Sensitivity	10 U/L
Linearity	1200 U/L

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	macro	*Semi-micro*
Working solution	1.0 ml	500 µl
Specimen	20 µl	10 µl

*Mix, r*ead 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

U/L = 8095 x ΔA 340 nm/min.
U/L = 15000 x ΔA 365 nm/min.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact ***Bioscien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean U/L	433	923	439	935
SD.	6.8	6.64	7.1	6.71
CV. %	1.57	0.71	1.62	0.79

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from ***Bioscien*** LDH reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.967.

Sensitivity
When run as recommended, the minimum detection limit of the 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.

EXPECTED VALUES

Serum/plasma	U/L	µkat/L
Adult	240-480	4.0-8.0
Children (7 – 12) years		
Males	< 580	< 9.65
Females	< 764	< 12.7
Premature	<1103	< 18.4

Temperature conversion factor is 0.5 (37 to 25 °C) And 0.67 (37 to 30 °C).

DYNAMIC RANGE

10 - 1200 U/L.

REFERENCES

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., JB Henery, editor, saunders, philadelphia,1979, pp 365-368.

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

EC REP

Medical Device Safety Service
MDSS GmbH
Schiffgr aben 41
30175 Hannover, Germany

CE