LDH / LD

(KINETIC) **(4+1)**

Ref. LDH - L D – 0520 (5 x 20 ml)

INTENDED USE

NS Biotec LDH reagent is intended for the in vitro quantitative determination of lactate dehydrogenase (EC 1.1.1.27) in human serum and plasma on both automated and manual systems.

CLINICAL SIGNIFICANCE

LDH is widely distributed in tissue, particularly, liver, muscle, and kidney. LDH in serum can be separated into five different isoenzymes based on their electrophoretic mobility. Each isoenzyme is a tetramer composed of two different subunits. These two subunits have been designated heart and muscle, based on their polypeptide chains. There are two homotetramers, LDH-1 (heart) and LDH-5 (muscle), and three hybrid isoenzymes. Elevated serum levels of LDH have been observed in a variety of disease states. The highest levels are seen in patients with megaloblastic anemia, myocardial infarction, disseminated carcinoma, leukemia, and trauma. Mild increases in LDH activity have been reported in cases of hemolytic anemia, muscular dystrophy, pulmonary infarction, hepatitis, nephortic syndrome, and cirrhosis.

ASSAY PRINCIPLE

LDH catalyze the reduction of pyruvate to lactate oxidizing reduced nicotinamide adenine dinucleotide (NADH) to NAD.

$$\text{Pyruvate} + \text{NADH} + H^+ \xleftrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

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

EXPECTED VALUES (at 37 oC)

Adults at 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 25oC) and 0.67 (37 30oC).

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the LDH results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

REAGENTS

R_1	Tris buffer pH 7.5	50 mmol/l
	Pyruvate	0.6 mmol/l
R_2	NADH	0.18 mmol/l

SPECIMEN

Use nonhaemolyzed 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 – 8oC ; 4 days at 20 – 25oC

Freezing of the samples is not recommended.

Reagent Preparation & Stability

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8^{0}C.

- Add 4 ml from R2 to one bottle of R1; mix gently.Or prepare the working solution according to the number of tests required by mixing 4 volumes of reagent 1 (R1) and 1 volume of reagent 2 (R2),e.g. 400 ml R1 +100 ml R2.

- **PROCEDURE**

- **Manual Procedure**

Wavelength 340 nm (334- 365 nm)
Cuvette 1 cm light path
Temperature 25, 30 or 37 ^{0}C
Zero adjustment against air
Specimen Serum or plasma

Pipette into test tube or cuvette	
Working solution	1000 µl
Serum or plasma	20 µl

Mix, incubate for 30 seconds, and start stopwatch simultaneously. Read again after exactly 1, 2, and 3 minutes.

Automated Procedure

User defined parameters for different auto analyzers are available upon request.

CALCULATION

Determine the change in absorbance per minute (ΔA/min) from the linear portion of the reaction curve and calculate the LDH activity by using the following formulae:

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

One international unit **(U)** is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

The general formula for converting ΔA/min into U/l is:

$$U/l = \frac{\Delta A/min \times TV \times 1000}{*\sum \times SV \times LP}$$

Where:

TV	Total reaction volume in ml
SV	Sample volume in ml
*∑	millimolar absorptivity of NADH
LP	Cuvette pathlength in cm
1000	Conversion of U/ml to U/l

***** millimolar absorptivity of NADH
at 334 nm= 6.18,
at 340 nm= 6.22, and
at 365 nm= 6.40

SENSITIVITY

The sensitivity is defined as the lower detection limit represents the lowest measurable LDH activity that can be distinguished from zero.

When run as recommended the sensitivity of this assay is 10 U/l

LINEARITY

When run as recommended, the assay is linear up to 1200 U/l

If result exceeds 1000 U/l or 16.67 µkat/l, specimen should be diluted 1+5 with 0.9% NaCl solution and reassayed. Multiply the result by 6.

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established LDH values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
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- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact NS Biotec Technical Services.

INTERFERING SUBSTANCES

The only acceptable anticoagulant is heparin and EDTA.

- **Bilirubin:**
  No significant interference from free or conjugated bilirubin up to a level of 29 mg/dl.
- **Drugs:**
  Youngs[13] in 1990 has published a comprehensive list of drugs and substances which may interfere with this assay.
- **Haemolysis:**
  Any erythrocyte contamination elevates results, since LDH activities in erythrocytes are 150 times higher than in normal sera.
- **Lipemia:**
  No interference from lipemia, measured as triglycerides, up to 877 mg/dl.

WARNING & PRECAUTIONS

NS Biotec LDH reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.

- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- Don't use the reagent if it is turbid or if the absorbance against water is greater than 0.8 at 405 nm.
- Reagent contains sodium azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
- Sodium azide reacts with lead or copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

BIBLIOGRAPHY

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3. **Kay, HD (1930):** J. Biol, Chem. 89: 235.

	Consult Instruction for Use
	Caution Consult Accompanying Documents
IVD	In Vitro Diagnostic Medical Device
	Temperature Limitation
	Manufacturer
EC REP	Authorized Representative In The European Community
REF	Catalogue Number
LOT	Batch Code
	Use By

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