LDH/LD

KINETIC

[P ⟶ L]

INTENDED USE

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

METHOD

Kinetic method according to the recommended reference method of DGKC[1]. Liquid stable double reagent.

BACKGROUND

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.

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

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

Adults

25°C	120 - 240	U/l
	2.0- 4.0	µkat/l
30°C	160 - 320	U/l
	2.66 - 5.33	µkat/l
37°C	230 - 460	U/l
	3.83- 7.66	µkat/l

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

- **Reagent Preparation & Stability**

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8°C.
Prepare the working solution by adding
one (1) volume of **R_2** to four (4) volumes of **R_1**.
Mix well, do not shake, protect from direct light.
The working solution is stable for 8 weeks at 2–8 °C if microbial contamination is avoided.

SPECIMEN

Serum, heparinized or EDTA plasma.

Separated serum or plasma should not remain at +15°C to +30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2 to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to –20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

PROCEDURE

- **Manual Procedure**

Wavelength 340 nm (334- 365 nm)
Cuvette 1 cm light path
Temperature 25, 30 or 37 °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:

$$\text{U/l} = 8095 \times \Delta\text{A 340 nm/min}$$

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:

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

Where:

TV	Total reaction volume in ml
SV	Sample volume in ml
*ε	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

- **Temperature correction**

Multiply the result by 1.34 if the assay performed at 25°C but is to be reported at 30°C.
Multiply the result by 2 if the assay performed at 25°C but is to be reported at 37°C.

Multiply the result by 1.52 if the assay performed at 30°C but is to be reported at 37°C.

Medical Device Safety Services
MDSS GmbH
Burckhardtstr. 1
30163 Hannover, Germany
Tel.: +49 511 6262 8630
Fax: +49 511 6262 8633

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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.
- 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 Vitro Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**
  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 & PRECAUTION

Vitro 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.

PERFORMANCE CHARACTERISTICS

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run	
Control	Level I	Level II
Number of samples	40	40
Mean (U/l)	360	485
SD (U/l)	6.1	6.4
CV (%)	1.7	1.4

	Between Day	
Control	Level I	Level II
Number of samples	40	40
Mean (U/l)	360	485
SD (U/l)	7	7.9
CV (%)	2.0	1.7

Method Comparison

Comparison studies were carried out using another similar commercially available LDH-P reagent. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:

Number of samples 40
Range of sample results 181 - 477 U/l
Mean of reference method results 263 U/l
Mean of LDH-P results 273 U/l
Slope 0.99
Intercept -1.57
Correlation coefficient 0.993

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 5 U/l or 0.08 μkat/l).

LINEARITY

When run as recommended, the assay is linear up to 1000 U/l or 16.67 μkat/l.

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

BIBLIOGRAPHY

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3. **Kay, HD (1930):** J. Biol, Chem. 89: 235.
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5. **Fugita, H (1939):** Uber die Mioro-bestimmung der Blutphosphatase. J Biochem (Japan) 30: 69.
6. **The committee on Enzymes of The Scandinavian Society for Clinical Chemistry and Clinical Physiology**

SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
	Batch code (Lot #)
	Catalog number
	Temperature limitation
	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
1321	5 X 10 ml
1322	10 X 10 ml
1323	4 X 20 ml
1324	3 X 50 ml
1325	10 X 20 ml

Manufactured in Egypt by:
Vitro Scient
www. vitroscient.com

Technical Support:
+202 26439699
info@vitroscient.com

orders:
+202 26439698
order@vitroscient.com

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