Lactate dehydrogenase (LDH)-Liquizyme (4+1) E.C.1.1.1.27.

REF: 283 001 (4 x 20 ml) 80 test
REF: 283 002 (10 x 10 ml) 100 test
REF: 283 003 (9 x 20 ml) 180 test
REF: 283 004 (4 x 60 ml) 240 test
REF: 283 005 (5 x 20 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 anaemia, disseminated carcinoma, leukemia, and trauma. Mild increases in LDH activity has been reported in cases of haemolytic anaemia, muscular dystrophy, pulmonary infarction, hepatitis, nephrotic syndrome, and cirrhosis.

Method

Kinetic ultraviolet method.

Assay Principle

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

\[
\text{Pyruvate + NADH + H}^+ \rightleftharpoons \text{L-Lactate + NAD}^+ 
\]

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

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 Coenzyme)

NADH > 0.18 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 inhale. 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

REF:283 001: add 4 ml from R2 to one bottle of R1; mix gently.
REF:283 002: add 2 ml from R2 to one bottle of R1; mix gently.
REF:283 003: add 4 ml from R2 to one bottle of R1; mix gently.
REF:283004: add 12 ml from R2 to one bottle of R1; mix gently.
REF:283 005: 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 µl R1 +100 µl R2.

Reagent Storage and Stability

All reagents are stable until expiration date stated on label when stored refrigerated at 2 - 8 °C. Working solution is stable for 2 months at 2 - 8 °C or 1 week 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 nonhaemolysed 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 (336 – 365 nm)
Optical path 1 cm
Assay type Kinetic
Direction decrease
Sample : Reagent Ratio e.g.: Reagent volume 1 ml
Sample volume 20 µl
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): Macro Semi-Micro
Working solution 1 ml 500 µl
Specimen 20 µl 10 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 formula

\[
U/L = \frac{\Delta A \times 340 \times 1000}{\Delta A \times \text{min}}
\]

U/L = 15000 x AU 365 mm/min.

Quality Control

Normal & abnormal control serum of known concentrations should be analyzed with each run.
Performance Characteristics

Precision
Within run (Repeatability)

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (U/L)</td>
<td>433</td>
<td>923</td>
</tr>
<tr>
<td>SD</td>
<td>6.8</td>
<td>6.64</td>
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<tr>
<td>CV%</td>
<td>1.57</td>
<td>0.71</td>
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Run to run (Reproducibility)

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<tr>
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<tr>
<td>Mean (U/L)</td>
<td>439</td>
<td>935</td>
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<tr>
<td>SD</td>
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<tr>
<td>CV%</td>
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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 x 6).

Interfering substances
Serum, plasma

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 (at 37 °C)

<table>
<thead>
<tr>
<th></th>
<th>240-480 U/L</th>
<th>(4.0- 8.0 µkat/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children (7-12 Years)</td>
<td>&lt; 580 U/L</td>
<td>(&lt; 9.65 µkat/L)</td>
</tr>
<tr>
<td>Female</td>
<td>&lt; 764 U/L</td>
<td>(&lt; 12.7 µkat/L)</td>
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<tr>
<td>Male</td>
<td>&lt; 1103 U/L</td>
<td>(&lt; 18.4 µkat/L)</td>
</tr>
<tr>
<td>Premature</td>
<td></td>
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Calculate for temperature conversion factor of 0.5 (37 → 25°C) and 0.67 (37 → 30°C).

Spectrum Diagnostics 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.

S66: dispose of this material and its container at hazardous waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

References

ORDERING INFORMATION

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<thead>
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