## LDH-L Reagent

### PRODUCT SUMMARY

<table>
<thead>
<tr>
<th><strong>Stability</strong></th>
<th>5 days at 2-8°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linear Range</strong></td>
<td>20 - 1000 U/L</td>
</tr>
<tr>
<td><strong>Specimen Type</strong></td>
<td>Serum</td>
</tr>
<tr>
<td><strong>Method</strong></td>
<td>Kinetic</td>
</tr>
<tr>
<td><strong>Reagent Preparation</strong></td>
<td>Add specified volume of distilled or deionised water.</td>
</tr>
</tbody>
</table>

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### SYMBOLS IN PRODUCT LABELLING

<table>
<thead>
<tr>
<th><strong>EC REP</strong></th>
<th><strong>Authorized Representative</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IVD</strong></td>
<td>For in vitro diagnostic use</td>
</tr>
<tr>
<td><strong>LOT</strong></td>
<td>Batch code/Lot number</td>
</tr>
<tr>
<td><strong>REF</strong></td>
<td>Catalogue number</td>
</tr>
<tr>
<td><strong>Temperature Limitation</strong></td>
<td>Use by/Expiration Date</td>
</tr>
</tbody>
</table>

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### INTENDED USE

This reagent is intended for the in vitro quantitative determination of LDH (L-Lactate: NAD oxidoreductase EC 1.1.1.27) in human serum on both manual or automated systems.

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### CLINICAL SIGNIFICANCE

The enzyme Lactate dehydrogenase (LDH) is concentrated in heart, kidney, liver, muscle and body tissues. Consequently, damage to these tissues results in increased serum levels of LDH. Elevated levels are associated with myocardial infarction, renal damage, hepatitis, anaemia’s, malignancies and muscular disease or damage. There are at least five forms of LDH separable by electrophoresis. The predominant form present varies with the tissue of origin, and therefore, has diagnostic value.

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### METHODOLOGY

Although the activity of LDH can be measured utilising pyruvate or lactate as a substrate, this reagent uses lactate and is based on the procedure of Gay, McComb and Bowers.

LDH catalyses the oxidation of lactate to pyruvate reducing nicotinamide adenine dinucleotide (NAD) to NADH. The activity of LDH can be determined by the rate of increase in absorbance at 340 nm as NADH is produced.

### REAGENT COMPOSITION

<table>
<thead>
<tr>
<th><strong>Active Ingredient</strong></th>
<th><strong>Concentration</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris Buffer</td>
<td>100 mmol/L</td>
</tr>
<tr>
<td>NAD</td>
<td>7 mmol/L</td>
</tr>
<tr>
<td>Lithium Lactate</td>
<td>50 mmol/L</td>
</tr>
<tr>
<td>KCl</td>
<td>120 mmol/L</td>
</tr>
<tr>
<td>pH 9.0 ± 0.1 at 20°C.</td>
<td></td>
</tr>
</tbody>
</table>

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### CAUTION

Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Flush with plenty of water when disposing.

### REAGENT PREPARATION

Reconstitute the reagent with the volume of distilled or deionised water stated on the vial label. Mix gently until dissolved.

### STABILITY AND STORAGE

**Prior to use:**
When stored refrigerated at 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

**Reconstituted Reagent:**
When stored capped at 2-8°C, the reagent is stable for at least 5 days.

### Indications of Reagent Deterioration:

- Turbidity, and/or
- Failure to recover control values within the assigned range.

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### SPECIMEN COLLECTION AND HANDLING

**Serum:** Use non-haemolysed serum.

**Plasma:** Not recommended.

**Storage:** LDH samples may be stored for at least 1 to 3 days at room temperature (18-25°C) and for at least 7 days at 4°C. Do not freeze the sample as this will destroy the liver isoenzyme.

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### ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyzer capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm.
- Analyzer specific consumables, eg: sample cups.
- Distilled or deionized water for reagent preparation and related equipment eg: pipettes.
- Normal and Abnormal assayed control material.

### ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

### SYSTEM PARAMETERS

| **Temperature** | 30°/37°C |
| **Wavelength** | 340 nm (334 - 365nm) |
| **Assay Type** | Rate/Kinetic |
| **Direction** | Increase |
| **Sample : Reagent Ratio** | 1 : 60 |
| **Delay/Lag Time** | 30 seconds |
| **Read Time** | 60 seconds |
| **Reagent Blank Limits** | Low 0.0 AU |
| **Linearity** | 20 - 1000 U/L |
| **Sensitivity** | 0.103 ±mA/min per U/L |

### CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

\[
\text{Activity in U/L} = \frac{\Delta \text{Abs/min} \times \text{Factor}}{6.3 \times \text{SV} \times \text{P}}
\]

Where:

- \( \Delta \text{Abs/min} \) = Total reaction volume in mL
- \( \text{SV} \) = Sample volume in mL
- \( 6.3 \) = millimolar absorption coefficient of NADH at 340nm
- \( \text{P} \) = Cuvette pathlength in cm.

**Example:**

\[
\Delta \text{Abs/min} = 0.015 \\
\text{Factor} = 9683 \\
\text{LDH} = 0.015 \times 9683 = 145 \text{ U/L}
\]
NOTES
1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. If the change in absorbance is greater than 0.10 /min, dilute with saline and re assay. Multiply the final result by the dilution factor.
3. Valid results depend on accurately calibrated instruments, timing and temperature control.
4. The milimolar absorption coefficient for NADH at 334 nm = 6.18 and at 365 nm = 3.40.
5. Unit conversion: U/L x 16.67 x 10⁻³ = µkat/L.

CALIBRATION
Not required. The rate of reaction is converted to U/L of activity by a calculation factor. Refer to the calculation section of this package insert.

QUALITY CONTROL
To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-
• At least every eight hours.
• When a new bottle of reagent is used.
• After preventative maintenance is performed or a critical component is replaced.
Control results falling above the upper limit or below the lower limit of the established range indicates the assay may be out of control.
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 Technical Services or the local distributor.

LIMITATIONS
1. Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out on a well maintained automated clinical chemistry analyzer. The following results were obtained:
   Haemoglobin: No interference from haemoglobin up to 500 mg/dL.
   Bilirubin: No interference from bilirubin up to 510 µmol/L (30 mg/dL).
   Lipaemia: No interference from lipaemia, measured as triglycerides, up to 11.4 mmol/L (1000 mg/dL).
2. Young DS⁴ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES⁵
At 37°C: 114 to 240 U/L
The quoted values should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.⁶

PERFORMANCE DATA
The following data was obtained using the LDH-L reagent on a well maintained automated clinical chemistry analyzer. Users should establish product performance on their specific analyzer used.

IMPRECISION
Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure.⁷

<table>
<thead>
<tr>
<th>LEVEL I</th>
<th>LEVEL II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (U/L)</td>
<td>120</td>
</tr>
<tr>
<td>CV (%) Within run</td>
<td>2.3</td>
</tr>
<tr>
<td>CV (%) Between day</td>
<td>3.2</td>
</tr>
</tbody>
</table>

ACCURACY
Comparison studies were carried out using another commercially available method as a reference. Serum samples were assayed and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs | 60 |
|------------------------|----|
Range of sample results | 85 - 696 U/L |
| Mean of reference method results | 169 U/L |
| Mean of LDH-L results | 172 U/L |
| Slope | 0.99 |
| Intercept | 4.4 U/L |
| Correlation coefficient | 0.997 |

LINEARITY
When run as recommended, the assay is linear between 20 and 1000 U/L (0.33 and 16.67 µkat/L).

SENSITIVITY
When run as recommended the sensitivity of this assay is 0.103 ΔmA/min per U/L.

REFERENCES

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Reorder Information
Catalogue No. TR20015
Configuration 20 x 20 mL