Lactate – Liquizyme

REF: 274 001 (5 x 20 ml) 100 test

Intended Use
Spectrum Diagnostics liquizyme Lactate reagent is intended for the in-vitro quantitative, diagnostic determination of lactate in human plasma and CSF on both automated and manual systems.

Background
Lactic acid, present in blood entirely as lactate, is an intermediary product of carbohydrate metabolism and is derived mainly from muscle cells and erythrocytes. The blood lactate concentration is affected by its production in muscle cells and erythrocytes and its rate metabolism in the liver. During exercise, blood lactate can increase up to ten times of normal levels. Under normal conditions, the ratio between lactate and pyruvate is constant (10:1). The liver can normally metabolize more lactate than is produced. In the case of decreased perfusion of the liver, however, removal of lactate by the liver may be significantly reduced. The amount of lactate in cerebrospinal fluid normally parallels blood levels. CSF lactate level is increased in bacterial meningitis, epilepsy, and intracranial hemorrhage. CSF lactate level may be an aid to distinguish between bacterial from viral meningitis.

Method
Enzymatic colorimetric method (LOX / PAP) with lactate oxidase and 4-aminoantipyrine.

Assay Principle
Lactate is oxidized to pyruvate and hydrogen peroxide (H₂O₂) by lactate oxidase (LOX). In the presence of peroxidase (POD), hydrogen peroxide reacts with 2,4,6-tribromo-3-hydroxybenzoic acid (THB) and 4-aminoantipyrine (4-AAP) to form a red quinoneimine dye.

\[
\begin{align*}
\text{Lactate} & \rightarrow \text{Pyruvate} \\
+ & \text{LOX} \\
+ & \text{O₂} \\
\rightarrow & \text{H₂O₂} \\
2\text{H₂O} + 4\text{-AAP} & \rightarrow \text{THB} \\
& \rightarrow \text{quinoneimine dye} \\
\end{align*}
\]

The color intensity of the formed red quinoneimine dye is directly proportional to the lactate concentration. It is determined by measuring the increase in absorbance at 546 nm.

Reagents

- **Standard lactate (ST)** 10 mg/dL
- **Reagent 1 (R1 Buffer)**
  - Tris buffer 100 mmol/L
  - 2,4,6-tribromo-3-hydroxybenzoic acid 2.0 mmol/L
  - 4-Amino antipyrine 0.8 mmol/L
- **Reagent 2 (R2 Enzyme)**
  - Lactate oxidase >20 U/L
  - Peroxidase >15 U/L
  - Sodium Azide 0.02 %

For further information, refer to the Lactate 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.

Reagent (R2) contains sodium azide which may react with copper or lead plumbing.

Reagent Preparation
Prepare working solution as following:

REF:274 001: add 2 ml from R2 to one bottle of R1; mix gently.
Or prepare the working solution according to the number of tests required by mixing 9 volumes of reagent 1 (R1) and 1 volume of reagent 2 (R2), e.g. 300 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 3 months at 2 – 8 °C or 1 week at 15 – 25 °C.

Deterioration
The working reagent is normally clear or pale pink. Do not use liquizyme lactate reagent if it is turbid or if the absorbance is greater than 0.1 at 546 nm.

Specimen Collection and Preservation
Plasma and CSF. Do not use serum specimens. Avoid icteric and haemolytic specimens. The only acceptable anticoagulants are fluoride/heparin and iodoacetate/heparin. Collection of satisfactory specimen for lactate analysis requires special procedures to prevent changes of lactate both while and after the specimen is drawn. The patient should be fasting and at complete rest and exercise of the arm or hand should be avoided before or during collection of the specimens. The collected blood should be cooled on ice immediately and separated from the cells within 15 minutes. Once the plasma is separated from the cells, lactate values are stable.

Use the CSF samples with addition of glycolysis inhibitor, e.g. sodium fluoride. Lactate in CSF is stable for 3 hours at 20 – 25 °C, for 24 hours at 4 – 8 °C, and for 2 months frozen at -20 °C, stable in plasma for 2 hours at 20 – 25 °C and 2 days at 4 – 8 °C.

System Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>546 nm</td>
</tr>
<tr>
<td>Optical path</td>
<td>1 cm</td>
</tr>
<tr>
<td>Assay type</td>
<td>End-point</td>
</tr>
<tr>
<td>Direction</td>
<td>Increase</td>
</tr>
<tr>
<td>Sample: Reagent Ratio</td>
<td>1:100</td>
</tr>
<tr>
<td>e.g: Reagent volume</td>
<td>1 ml</td>
</tr>
<tr>
<td>Sample volume</td>
<td>10 µl</td>
</tr>
<tr>
<td>Temperature</td>
<td>37 °C or 15 – 25 °C</td>
</tr>
<tr>
<td>Zero adjustment</td>
<td>Reagent blank</td>
</tr>
<tr>
<td>Incubation time</td>
<td>5 minutes at 37 °C or 10 minutes at 15 – 25 °C</td>
</tr>
<tr>
<td>Reagent Blank Limits</td>
<td>Low 0.00 AU</td>
</tr>
<tr>
<td></td>
<td>High 0.25 AU</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.3 mg/dL (0.033 mmol/L)</td>
</tr>
<tr>
<td>Linearity</td>
<td>90 mg/dL (9.99 mmol/L)</td>
</tr>
</tbody>
</table>

**Procedure**

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>10 µl</td>
<td>10 µl</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

Mix and incubate for 5 minutes at 37 °C or 10 minutes at 15 – 25 °C. Measure absorbance of specimen (A<sub>specimen</sub>) and standard (A<sub>standard</sub>) against reagent blank within 30 minutes.

**Symbols in Product Labelling**

- **EC REP** Authorised Representative
- **LOT** Batch Code/Lot number
- **Consult instructions for use** Manufactured by
Calculation
Lactate conc. (mg/dL) = \( \frac{A_{\text{specimen}}}{A_{\text{standard}}} \times 10 \)

Quality Control
Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Methods Comparison
A comparison between Spectrum Diagnostics Lactate reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.992 was obtained.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 0.3 mg/dL (0.033 mmol/L).

Linearity
The reaction is linear up to lactate concentration of 90 mg/dL (9.99 mmol/L), specimens showing higher concentration should be diluted 1+1 using physiological saline, reassayed and the result multiplied by two.

Interfering substance
Plasma
Haemolysis
Haemoglobin levels higher than 2.5 g/L (0.16 mmol/L) increase the apparent lactate concentration significantly.

Icterus
Bilirubin levels higher than 4.0 mg/dL (68 mmol/L) decrease apparent lactate concentration significantly.

Lipemia
No significant interference.

Ascorbic acid
Physiological ascorbic acid concentrations do not interfere with the test. Ascorbic Acid levels higher than 5 mg/dL (284 mmol/l) decrease the apparent lactate concentration significantly.

Expected Values
<table>
<thead>
<tr>
<th>Type</th>
<th>Venous</th>
<th>Arterial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>4.5 – 19.8 mg/dL</td>
<td>0.5 – 2.2 mmol/L</td>
</tr>
<tr>
<td>Arterial</td>
<td>4.5 – 14.4 mg/dL</td>
<td>0.5 – 1.6 mmol/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type</th>
<th>Adult</th>
<th>Neonates</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>10 – 22 mg/dL</td>
<td>1.1 – 2.4 mmol/L</td>
</tr>
<tr>
<td>Neonates</td>
<td>10 – 60 mg/dL</td>
<td>1.1 – 6.7 mmol/L</td>
</tr>
</tbody>
</table>

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
0.3 – 90 mg/dL (0.033 – 9.99 mmol/L).

Waste Disposal
This product is made to be used in professional laboratories. Please consult local regulations for a correct 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. refer to special instructions/safety data sheets.