| COD 11580<br>1 x 50 mL | COD 11581<br>1 x 200 mL |
|---|---|
| STORE AT 2-8ºC | |
| Reagents for measurement of LDH concentration<br>Only for *in vitro* use in the clinical laboratory | |

# LACTATE DEHYDROGENASE (LDH)

BioSystems

CE

## LACTATE DEHYDROGENASE (LDH)

PYRUVATE

## PRINCIPLE OF THE METHOD

Lactate dehydrogenase (LD or LDH) catalyzes the reduction of pyruvate by NADH, to form lactate and $NAD^+$. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm[1,2].

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

## CONTENTS

| | COD 11580 | COD 11581 |
|---|---|---|
| A. Reagent | 1 x 40 mL | 1 x 160 mL |
| B. Reagent | 1 x 10 mL | 1 x 40 mL |

## COMPOSITION

A. Reagent: Tris 100 mmol/L, pyruvate 2.75 mmol/L, sodium chloride 222 mmol/L, pH 7.2

B. Reagent: NADH 1.55 mmol/L, sodium azide 9.5 g/L.

*WARNING: H302: Harmful if swallowed. EUH031: Contact with acids liberates toxic gas. P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P330: Rinse mouth.*

## STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagent: Presence of particulate material, turbidity, absorbance of the blank lower than 1.200 at 340 nm (1 cm cuvette).

## WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines. Any serious incident that might occur in relation to the device shall be reported to BioSystems S.A.

## REAGENT PREPARATION

Working Reagent. Pour the contents of the Reagent B into the Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 months at 2-8ºC.

## ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37ºC and able to read at 340 nm.

– Cuvettes with 1 cm light path.

## SAMPLES

Serum or plasma collected by standard procedures. Serum or plasma must be separated from the clot as soon as possible. In plasma ensure that the centrifugation is adequate to remove platelets. Do not use hemolysed samples.

Lactate dehydrogenase in serum or plasma is stable for 2 days at room temperature and for 24 hours at 2-8ºC. Use heparin as anticoagulant.

## PROCEDURE

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Note 1)

| | |
|---|---|
| Working Reagent | 1.0 mL |
| Sample | 20 µL |

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. After 30 seconds, record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute ( ΔA/min).

## CALCULATIONS

The LDH concentration in the sample is calculated using the following general formula:

$$\Delta A/\text{min} \times \frac{Vt \times 10^6}{\varepsilon \times l \times Vs} = \text{U/L}$$

The molar absorbance (ε) of NADH at 340 nm is 6300 and the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.02, the sample volume (Vs) is 0.02 and 1 U/L are 0.0166 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

| | |
|---|---|
| ΔA/min | x 8095 = U/L<br>x 135 = µkat/L |

## REFERENCE VALUES

| Reaction temperature | Adults U/L | Adults µKat/L |
|---|---|---|
| 37ºC[1] | 207 - 414 | 3.40 - 6.80 |

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

## QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

## METROLOGICAL CHARACTERISTICS

– Detection limit: 4.7 U/L = 0.078 µkat/L.

– Linearity limit: 1250 U/L = 20.92 µkat/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

| Mean Concentration | CV | n |
|---|---|---|
| 324 U/L = 5.40 µkat/L | 3.9 % | 20 |
| 1029 U/L = 17.15 µkat/L | 2.3 % | 20 |

– Reproducibility (run to run):

| Mean Concentration | CV | n |
|---|---|---|
| 324 U/L = 5.40 µkat/L | 6.6% | 25 |
| 1029 U/L = 17.15 µkat/L | 3.3 % | 25 |

– Sensitivity: 0.123 ΔmA·L/U·min = 7.41 ΔmA ·L/µkat·min.

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Interferences: Hemolysis interferes due to the high lactate dehydrogenase concentration in red cells. Lipemia (triglycerides < 10 g/L) and bilirubin (< 20 mg/dL) do not interfere. Other drugs and substances may interfere[3].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

## DIAGNOSTIC CHARACTERISTICS

Lactate dehydrogenase is present in all cells of the body but its higher concentrations are found in liver, heart, kidney, skeletal muscle and erythrocytes.

Total LDH concentration in serum or plasma is increased in patients with liver disease, renal disease, myocardial infarction, many malignant diseases, progresive muscular dystrophy and almost any cause of hemolysis[4,5].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

## NOTES

1. This reagent may be used in several automatic analysers. Instructions for many of them are available on request.

## BIBLIOGRAPHY


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