COD 11586 1 x 50 mL	COD 11587 1 x 200 mL
STORE AT 2-8ºC	
Reagents for measurement of LDH concentration Only for *in vitro* use in the clinical laboratory	

LACTATE DEHYDROGENASE (LDH) - IFCC

BioSystems
REAGENTS & INSTRUMENTS

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IFCC

PRINCIPLE OF THE METHOD

Lactate dehydrogenase (LD or LDH) catalyzes the oxidation of lactate by NAD^+, to form pyruvate and NADH. The catalytic concentration is determined from the rate of increase of NADH, measured at 340 nm[1,2,3].

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

CONTENTS

	COD 11586	COD 11587
A. Reagent	1 x 40 mL	1 x 160 mL
B. Reagent	1 x 10 mL	1 x 40 mL

COMPOSITION

A. Reagent: N-Methyl-D-glucamine 0.406 mol/L, lactate 62.5 mmol/L, pH 9.4

B. Reagent: NAD^+ 25 mmol/L.

WARNING: H317: May cause an allergic skin reaction. P302+P352: IF ON SKIN: Wash with plenty of soap and water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention.

For further warnings and precautions, see the product safety data sheet (SDS).

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 over 0.600 at 340 nm (1 cm cuvette).

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 days at 2-8ºC.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 30 or 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. 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	25 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 1.025, the sample volume (Vs) is 0.025 and 1 U/L are 0.01667 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

ΔA/min	x 6508 = U/L x 108 = µkat/L

REFERENCE VALUES

Reaction temperature	Adults U/L	Adults µKat/L
30ºC	83 - 156	1.38 - 2.59
37º C[2]	132 - 248	2.20 - 4.13

Values at 30ºC are obtained from those at 37ºC by using a conversion factor[2].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: 6.2 U/L = 0.103 µkat/L.

– Linearity limit: 1500 U/L = 33.33 µkat/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean Concentration	CV	n
164 U/L = 2.73 µkat/L	0.9 %	20
258 U/L = 4.30 µkat/L	0.7 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
164 U/L = 2.73 µkat/L	1.4 %	25
258 U/L = 4.30 µkat/L	1.3 %	25

– Sensitivity: 0.154 ΔmA·L/U·min = 9.26 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[4].

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, progressive muscular dystrophy and almost any cause of hemolysis[5,6].

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

1. Lorentz K, Klauke R, Schimidt E. Recommendation for the determination of the catalytic concentration of lactate dehydrogenase at 37ºC. *Eur J Clin Chem Clin Biochem* 1993;31:897-899.
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4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
5. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
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