COD 11580 1 x 50 mL	COD 11581 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)

BioSystems
REAGENTS & INSTRUMENTS

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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.

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 lower than 1.200 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 2 months at 2-8ºC.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 25, 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	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 x 135 = µkat/L

REFERENCE VALUES

Reaction temperature	Adults U/L	Adults µKat/L
25ºC	105-210	1.70-3.50
30ºC[2]	140-280	2.30-4.70
37ºC[1]	207-414	3.40-6.80

Values at 25ºC are obtained from those at 30ºC by using a conversion factor.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

1. Sociedad Española de Química Clínica, Comité Científico, Comisión de Enzimas. Método recomendado para la determinación en rutina de la concentración catalítica de lactato deshidrogenasa en suero sanguíneo humano. Quim Clin 1989; 8: 57-61.
2. Scientific Commitee. Recommandations pour la mesure de la concentration catalytique de la lactate deshidrogenase dans le serum humain a 30°C. Ann Biol Clin 1982; 40: 87-164.
3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
4. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
5. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.