LDH BR CE

CONTENTS			
REF	1141010	LDH BR	2 x 50 mL
For *in vitro* diagnostic use only			

LDH BR

SFBC
UV enzymatic method
KINETIC

PRINCIPLE

Lactate dehydrogenase (LD/LDH) catalyzes the reduction of pyruvate to lactate (P-L) in the presence of reduced nicotinamide adenine dinucleotide (NADH) at pH 7.5.
The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD^+ proportional to the activity of LD present in the sample.

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow[\text{pH 7.5}]{\text{LD/LDH}} \text{L-Lactate} + NAD^+$$

The method follows the proposed optimised formulation of the SFBC.[1]

REAGENT COMPOSITION

R1 **LDH substrate.** TRIS buffer 100 mmol/L pH 7.5, pyruvate 2.75 mmol/L, sodium chloride 222 mmol/L.

R2 **LDH coenzyme.** NADH 1.55 mmol/L.

STORAGE AND STABILITY

Store at 2-8ºC.
The Reagents are stable until the expiry date stated on the label.

REAGENT PREPARATION

Working reagent. Mix 4 mL of **R1** + 1 mL of **R2**. Stable for 2 months at 2-8ºC. Protected from light.
Discard the reagent if presents an absorbance below 1.200 at 340 nm against distilled water or if it fails to recover the declared values of control sera.

SAMPLES

Serum free of hemolysis separated from the cells as soon as possible after collection. The use of heparin and citrate as anticoagulants have been reported to falsely elevate LD activity.
Freezing results in loss of activity of the enzyme.

INTERFERENCES

- Hemolysis results in the release of LD from red cells into the body fluid, giving falsely high results.
- Hiperbilirubinemia does not affect the LD assay.
- High concentrations of urea inhibit the enzyme.[2]

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 30/37ºC, capable to read at 340 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Preincubate working reagent, samples and controls to reaction temperature 30/37ºC.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

Reaction temperature	30/37ºC
Working reagent	1.0 mL
Sample or control	20 µL

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
5. Incubate for 30 seconds and record initial absorbance reading.
6. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.
7. Calculate the difference between absorbances.
8. Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

CALCULATIONS

U/L = ΔA/min x 8095

Samples with ΔA/min exceeding 0.150 at 340 nm should be diluted 1:10 with saline and assayed again. Multiply the results by 10.

If results are to be expressed as SI units apply:
U/L x 0.01667 = µkat/L

CS/00/034
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REFERENCE VALUES[1]

Serum

Temperature	37ºC	30ºC
Adults	207-414 U/L (3.40-6.80 µKat/L)	140-280 U/L (2.30-4.70 µKat/L)

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **BC600** HUMAN MULTISERA NORMAL
Borderline level of LDH. Assayed.

REF **BC650** HUMAN MULTISERA ABNORMAL
Elevated level of LDH. Assayed.

CLINICAL SIGNIFICANCE[2,3]

The enzyme activity found in circulation is a mixture of five isoenzymes. Each organ has a characteristic isoenzyme profile. Leakage of these isoenzymes from a diseased organ results in the elevation of total serum LD.

I*ncreased* levels become evident 8-12 hours after myocardial infarction reaching a maximum 4-5 days later. Elevated values in serum are also encountered in cases of pulmonary embolism and in about one third of patients with renal disease, specially those with pyelonephritis or tubular necrosis. In toxic hepatitis with jaundice, Hodking´s disease and abdominal and lung cancers elevations are specially high.

Moderate increases are also observed in cases of liver disease, megaloblastic and pernicious anemia and progressive muscular dystrophy.

Decreases are not important clinically.

ANALYTICAL PERFORMANCE

- ***Linearity.*** Up to 1200 U/L
- ***Precision***

U/L	Within-run			Between-run		
Mean	281	1.65	1130	281	430	980
SD	1.3	2.42	2.64	2.3	2.71	3.06
CV%	0.46	0.52	0.23	0.82	0.63	0.31
N	10	10	10	8	8	8

Replicates: 10 for each level. Replicates: 8 for each level
Instrument: CECIL CE 2001 for 4 days.

- ***Sensitivity.*** Using this reagent and method an ΔA/min of 0.00´ read at 340 nm is equivalent to 10 U/L of LDH activity.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:
N = 25 r = 0.997 y = 1.07x – 2.410

REFERENCES

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3. Tietz. N.W. Clinical Guide to Laboratory Tests, 3rd Edition. W.B. Saunders Co. Philadelphia, PA. (1995).

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