

LDH BR 

CONTENTS			
<b>REF</b>	1141010	LDH BR	2 x 50 mL
For <i>in vitro</i> diagnostic use only			

## LDH BR

SFBC

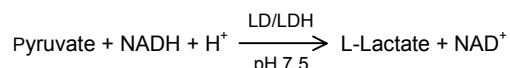
UV enzymatic method

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<sup>+</sup> proportional to the activity of LD present in the sample.




The method follows the proposed optimised formulation of the SFBC.<sup>1</sup>

## 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 2-8°C.

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 2-8°C. 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.<sup>2</sup>

## MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 30/37°C, 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 30/37°C.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

Reaction temperature	30/37°C
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

$$\text{U/L} = \Delta\text{A}/\text{min} \times 8095$$

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:

$$\text{U/L} \times 0.01667 = \mu\text{kat/L}$$



CS/00/034

## REFERENCE VALUES<sup>1</sup>

Serum

Temperature	37°C	30°C
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<sup>2,3</sup>

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.

*Increased* 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	SD	CV%	Mean	SD	CV%
281	1.65	1.3	0.46	281	2.3	0.82
1130	1.65	1.3	0.46	430	2.71	0.63
980	1.65	1.3	0.46	980	3.06	0.31
	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  $\Delta A/\text{min}$  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

1. Commission Enzymologie de la Société Française de Biologie Clinique. Ann. Biol. Clin. 40: 123-128 (1982).
2. Young, D.S. Effects of Drugs on Clinical Laboratory Tests. 4<sup>th</sup> Edition. AACC Press (1995).
3. Tietz. N.W. Clinical Guide to Laboratory Tests, 3<sup>rd</sup> Edition. W.B. Saunders Co. Philadelphia, PA. (1995).

B1141-1/0312  
R1.ing



CS/00/034