



LACTATE DEHYDROGENASE (LDH)

(According to SFBC)

UV method

E.C.1.1.1.27.

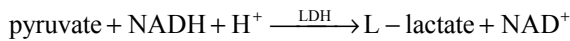
Cat.No. 101-0166 Size 20 x 2.5 ml

Cat.No. 101-0015 Size 10 x 10 ml

Cat.No. 101-0054 Size 4 x 50 ml

PRINCIPLE:

LDH catalyzes the reduction of pyruvate to L-lactate with concomitant oxidation of NADH₂ to NAD. Since the oxidation of NADH₂ is directly proportional to the reduction of pyruvate in equimolar amounts, the LDH activity can be calculated from the rate of decrease in absorbance at 340 nm (334 nm or 365 nm).



SAMPLE:

Serum, heparinized or EDTA plasma (avoid hemolysis).

REAGENTS:

- Reagent 1

Tris buffer, pH 7.5	80 mmol/L
NaCl	200 mmol/L
Pyruvate	1.60 mmol/L
- Reagent 2 , Coenzyme reagent

NADH	0.20 mmol/L
------	-------------

Store at +2 °C to +8 °C.

PREPARATION OF REAGENTS:

Dissolve reagent 2 with the corresponding volume of reagent 1 (buffer).

Working reagent is stable 21 days at +2 °C to +8 °C or 3 days at +15°C to +25 °C.

PROCEDURE:

Wavelength: 340 nm, 334 nm, 365 nm
 Cuvette: 1 cm light path
 Temperature: 30 °C, 37 °C
 Zero: air or distilled water

Pipette into cuvette	Micro	Semi	Macro
Working reagent	2500 µl	1000 µl	500 µl
Serum	100 µl	40 µl	20 µl
Mix, read initial absorbance after 30 sec, and at the same time start the stopwatch. Repeat reading after exactly 1, 2 and 3 min. Calculate ΔA/min			

CALCULATION:

$$\frac{\Delta A / \text{min} \times 10^6 \times \text{TV}}{6.3 \times 10^3 \times 1 \times \text{V}} = \Delta A / \text{min} \times F = \frac{U}{1}$$

Where is :

- ΔA = change in absorbance
 Min = minute
 6.3 x 10³ = molar absorptivity of NADH at 340 nm
 10⁶ = conversion of mol to µmol
 L = light path in cm
 TV = total reaction volume in ml
 V = sample volume in ml

Obtain the activity of LDH in the sample calculate as follows:

$$\Delta A_{334 \text{ nm}} / \text{min} \times 4207 = \text{U/L LDH}$$

$$\Delta A_{340 \text{ nm}} / \text{min} \times 4127 = \text{U/L LDH}$$

$$\Delta A_{365 \text{ nm}} / \text{min} \times 7647 = \text{U/L LDH}$$

$$\text{U/L} \times 16.67 = \text{nkat/L}$$

EXPECTED VALUES:

	30 °C	37 °C	Units
LDH	136 - 276 2267 - 4600	226 - 451 3767 - 7518	U/L nkat/L

LINEARITY:

up to 900 U/L (15000 nkat/L)

NOTE:

- If the absorbance change per minute ≥ 0.200 at 340 nm and 334 nm or ≥ 0.110 at 365 nm, dilute the sample 1:10 with physiological solution and reassay (result x 10). Avoid haemolysis as it interferes with assay.
- Solution buffer contains sodium azide. Avoid ingestion or contact with skin or mucous membranes.
- Sera with very high activities may show a very low initial absorbance, if the NADH₂ is partially consumed before taking the first reading. In this case repeat the analysis with sample diluted 1:10 (result x 10).

QUALITY CONTROL:

CONTRO-N 20 x 5 ml Cat. No. 101-0083
 CONTRO-P 20 x 5 ml Cat. No. 101-0084

REFERENCE:

- Bergmeyer, HU. J.Clin.Chem.Clin. Biochem 13, 507 (1975).
- Howell, BF. et al Clin.Chem. 25, 269 (1979)