

Manual Procedure

Cat. No. 17370 R1 1 x 40 ml
For 50 tests R2 1 x 10 ml

LDH-P

Kinetic UV method

Liquid Reagents

Teas principle

Kinetic determination of the lactate dehydrogenase according to the following reaction:



Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate to lactate with simultaneous oxidation of NADH to NAD. The rate of NADH oxidation can be measured as a decrease in absorbance at 340nm.

This rate is directly proportional to LDH activity in serum.

Concentrations in the test

| | | |
|-------------------------|-----|--------|
| Reagent R1 | | |
| TRIS | 80 | mmol/L |
| Pyruvate | 1.9 | mmol/L |
| Sodium chloride | 20 | mmol/L |
| Non-reactive stabilizer | | |
| Reagent R2 | | |
| NADH | 1.3 | mmol/L |
| Preservative | | |

Stability and preparation of working reagent

Reagent R1: liquid.

Reagent R2: liquid.

All reagents are stable up to expiry date given on the label when stored at +2 → +8 °C.

Working Reagent: (4+1)

Mix 4 volumes of bottle R1 with 1 volume of bottle R2.

Avoid direct exposure to light.

Stability: 4 weeks at 2 - 8 °C.

Specimen collection and handling

1. Non-hemolyzed serum, heparinized or EDTA plasma.
2. The serum or plasma should be separated from the erythrocytes promptly. Red cells contain large concentration of LDH.
3. LDH in serum is reported stable for 2 - 3 days at 20 - 25 °C, or for 7 days at 2 - 8 °C.
4. Don't expose the serum to high temperatures (37°C) as this may inactivate thermolabile LDH isoenzymes.

Calibrator

MediCal U Cat. No. 15011

Quality control

Meditrol N Cat. No. 15171

Meditrol P Cat. No. 15181

Procedure

| | |
|-------------------|--------------------------------|
| Wavelength | Hg 340 nm (334 - 365 nm) |
| Spectrophotometer | 340 nm |
| Cuvette | 1 cm light path |
| Temperature | 37°C |
| Measurement | against air or distilled water |
| Reaction | kinetic – decrease |

Assay: Incubate Working Reagent at 37 °C before use:

| | |
|--|---------|
| Sample | 20 µl |
| Working Reagent | 1000 µl |
| Mix, incubate for 30 sec. at 37 °C, then read change in the absorbance per 1 min. for 3 min. | |
| Determine the mean absorbance change per 1 min (ΔA/min). | |

Calculation

LDH-P activity (U/L) = (ΔA /min.) X Factor

Factor

| | | | |
|-----------------|--------|--------|--------|
| Wavelength | 334 nm | 340 nm | 365 nm |
| Factors at 37°C | 8252 | 8095 | 15000 |

Note: It is recommended that each laboratory (as per instrument performance) could determine its own factor (F) by the use of a calibrator according to the following formula:

$$F = \frac{\text{Conc. Calibrator}}{\Delta/\text{min Calibrator}}$$

Linearity

Up to 1200 U/L.

If the result exceeds 1200 U/L, repeat the test using diluted sample (1+2) with sodium chloride solution (0.9 %) and multiply the result by 3.

Interferences

1. Red cells contain large concentrations of LDH, hemolysis will cause falsely elevated values.
2. Certain drugs and substances affect LDH activity. See Young, *et al.*

Precautions

The reagents contain sodium azide as a preservative. Don't ingest. Avoid skin and eye contact. Sodium azide may react with lead and copper plumbing mixtures giving rise to explosive metal azides. Flush with large volumes of water when disposing of the reagent.

Reference range

| | | | |
|---------------|-------|--------|-----|
| 1 d. | | < 1327 | U/L |
| 2 – 5 d. | | < 1732 | U/L |
| 6 d. – 6 mth. | | < 975 | U/L |
| 7 – 12 mth. | | < 1100 | U/L |
| 1 – 3 yr. | | < 850 | U/L |
| 4 – 6 yr. | | < 615 | U/L |
| 7 – 12 yr. | women | < 580 | U/L |
| | men | < 764 | U/L |
| 13 – 17 yr. | women | < 436 | U/L |
| | men | < 683 | U/L |
| Adults | | < 480 | U/L |

References

1. Clin. Chem. Clin. Biochem. 8, 658 (1970), 1, 1820 (1972).
2. Ann. Biol. Clin., 40 (1982), 123.
3. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACCC Press, Washington, D.C.
4. Working Group on Enzymes. Proposal of standard methods for the determination of enzyme catalytic concentrations in serum and plasma at 37° C. IV. Lactate dehydrogenase. Eur J Clin Chem Clin Biochem 1992, 30: 787 - 92.
5. Lorentz K, Klauke R, Schmidt E. Recommendation for the determination of the catalytic concentration of lactate dehydrogenase at 37 °C. Eur J Clin Chem Clin Biochem 1993, 31: 897 - 9.
6. Deutsche Gesellschaft für Klinische Chemie. Recommendations of the German Society for Clinical Chemistry. J Clin Chem Clin Biochem 1972, 10 : 182 - 93.