

# Liquick Cor-LDH

## DIAGNOSTIC KIT FOR DETERMINATION OF LACTATE DEHYDROGENASE ACTIVITY



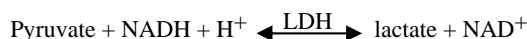
|                        |                |
|------------------------|----------------|
| <b>Kit name</b>        | <b>Cat. No</b> |
| Liquick Cor-LDH 500    | 1-315          |
| Liquick Cor-LDH "bulk" | 1-284          |

### INTRODUCTION

Lactate dehydrogenase (LDH, LD) is intracellular enzyme occurred in all tissues. LDH catalyzes the reversible conversion of lactate to pyruvate using  $\text{NAD}^+$  as a cofactor. LD is a tetramer containing two possible forms of subunits: H and M. The result is five isoenzymes termed LD-1 ( $\text{H}_4$ ) through LD-5 ( $\text{M}_4$ ). The isoenzymes are present in different proportion in each tissue and have different electrophoresis mobility, what is very useful for diagnostic.

### METHOD PRINCIPLE

Optimized kinetic method of Deutsche Gesellschaft für Klinische Chemie (DGKC).



The rate of absorbance changing at  $\lambda=340$  nm is directly proportional to lactate dehydrogenase activity.

### REAGENTS

| Package | Liquick Cor-LDH<br>500 | Liquick Cor-LDH<br>"bulk" |
|---------|------------------------|---------------------------|
| 1-LDH   | 3 x 400 ml             | --*                       |
| 2-LDH   | 1 x 300 ml             | --*                       |

\*reagent volume is printed on the label.

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 8 weeks on board the analyser at 2-10°C. Protect from light and avoid contamination!

### Working reagent preparation and stability

Assay can be performed with use of separate 1-LDH and 2-LDH reagents or with use of working reagent. For working reagent preparation mix gently 4 parts of 1-LDH with 1 part of 2-LDH. Avoid foaming!

Stability of working reagent: 5 days at 2-8°C  
24 hours at 15-25°C

Protect from light and avoid contamination!

### Concentrations in the test

|                           |             |
|---------------------------|-------------|
| phosphate buffer (pH 7.5) | 50 mmol/l   |
| pyruvate                  | 0.6 mmol/l  |
| NADH                      | 0.25 mmol/l |

### Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents contain sodium azide (< 0.1%) as a preservative. Avoid contact with skin and mucous membranes.
- The reagents are usable when absorbance of working reagent is higher than 1.000 (read against distilled water, wavelength  $\lambda=340$  nm, cuvette l=1 cm, at temp. 25°C).

### ADDITIONAL EQUIPMENT

- automatic analyzer or photometer able to read at 340 nm (Hg 334 nm, 365 nm);
- thermostat at 25°C or 37°C;
- general laboratory equipment;

### SPECIMEN

Serum, heparinized plasma free from hemolysis.  
Do not use hemolyzed blood or serum because erythrocytes contain 150 times more LDH activity than serum.

As an anticoagulant for plasma preparation use heparin lithium or ammonium salt.

LDH activity is unstable and is rapidly lost during storage.

Specimens can be stored up to 4 hours at 15-25°C or 1-2 days at 2-8°C.

Nevertheless it is recommended to perform the assay with freshly collected samples!

### PROCEDURE

These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analysers. Applications for them are available on request.

### Manual procedure

|             |                            |
|-------------|----------------------------|
| wavelength  | 340 nm (Hg 334 nm, 365 nm) |
| temperature | 25°C/37°C                  |
| cuvette     | 1 cm                       |

### Sample Start method

Pipette into the cuvette:

|                 |              |
|-----------------|--------------|
| working reagent | 1000 $\mu$ l |
|-----------------|--------------|

Bring up to the temperature of determination. Then add:

|        |  |
|--------|--|
| sample | 20 $\mu$ l (temp. 25°C)<br>or<br>10 $\mu$ l (temp. 37°C) |
|--------|--|

Mix and incubate at adequate temperature. After about 1 min. read the absorbance against air or water. Repeat the reading after exactly 1, 2 and 3 minutes. Calculate the mean absorbance change per minute ( $\Delta A/\text{min.}$ ).

### Calculation

LDH activity [U/l] =  $\Delta A/\text{min.} \times F$

F value depends on the used wavelength:

| $\lambda$ | 25°C  | 37°C  |
|-----------|-------|-------|
| 340 nm    | 8095  | 16030 |
| 334 nm    | 8250  | 16345 |
| 365 nm    | 15000 | 29705 |

### Reagent Start method

The determination can be also performed with use of separate 1-LDH and 2-LDH reagents.

Pipette into the cuvette:

|       |              |
|-------|--------------|
| 1-LDH | 1000 $\mu$ l |
|-------|--------------|

Bring up to the temperature of determination. Then add:

|        |  |
|--------|--|
| sample | 20 $\mu$ l (temp. 25°C)<br>or<br>10 $\mu$ l (temp. 37°C) |
|--------|--|

Mix well, incubate for 1-5 min. Then add:

|       |             |
|-------|-------------|
| 2-LDH | 250 $\mu$ l |
|-------|-------------|

Mix well, perform measurement as described for Sample Start method.

### Calculation

LDH activity [U/l] =  $\Delta A/\text{min.} \times F$

F value depends on the used wavelength:

| $\lambda$ | 25°C  | 37°C  |
|-----------|-------|-------|
| 340 nm    | 10080 | 20000 |
| 334 nm    | 10275 | 20390 |
| 365 nm    | 18675 | 37060 |

## REFERENCE VALUES <sup>4</sup>

|                |               |                    |
|----------------|---------------|--------------------|
| serum / plasma | 37°C          | 37°C               |
| adults         | 225 – 450 U/l | 3.75 – 7.50 µkat/l |

It is recommended for each laboratory to establish its own reference ranges for local population.

## QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.

For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) or LEVEL 2 (Cat. No 5-175; 5-177) is recommended.

The calibration curve should be prepared every 8 weeks, with change of reagent lot number or as required e.g. quality control findings outside the specified range.

## PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using the automatic analyser Biolis 24i Premium. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 20.1 U/l (0.36 µkat/l).
- **Linearity:** up to 2000 U/l (33.3 µkat/l).  
If LDH activity in tested sample 2000 U/l dilute the sample with 0.9% NaCl in the ratio of 1 to 9 and repeat the assay, multiply the result by 10.
- **Specificity / Interferences**  
Haemoglobin up to 5 g/dl, bilirubin up to 20 mg/dl, ascorbate up to 62 mg/l and triglycerides up to 1000 mg/dl do not interfere with the test.

- **Precision**

| Repeatability (run to run)<br>n = 20 | Mean<br>[U/l] | SD<br>[U/l] | CV<br>[%] |
|--------------------------------------|---------------|-------------|-----------|
| level 1                              | 317.41        | 3.40        | 1.07      |
| level 2                              | 784.04        | 9.78        | 1.25      |

| Reproducibility (day to day)<br>n = 80 | Mean<br>[U/l] | SD<br>[U/l] | CV<br>[%] |
|--|---------------|-------------|-----------|
| level 1                                | 312.47        | 3.26        | 1.04      |
| level 2                                | 782.43        | 7.43        | 0.95      |

- **Method comparison**

A comparison between LDH values for samples obtained on Biolis 24i Premium (y) and obtained on COBAS INTEGRA 400 (x) using 70 samples gave following results:

$$y = 0.9227 x + 21.385 \text{ U/l};$$

$$R = 0.9952 \quad (R - \text{correlation coefficient})$$

## WASTE MANAGEMENT

Please refer to local legal requirements.

## LITERATURE

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