

Liquick Cor-LDH

	(EN)	Cat. No
Kit name		
Liquick Cor-LDH mini		1-298
Liquick Cor-LDH 30		1-239
Liquick Cor-LDH 500		1-315

INTENDED USE

Diagnostic kit for determination of lactate dehydrogenase activity intended to use both for manual assay (Sample Start and Reagent Start method) and in several automatic analysers.

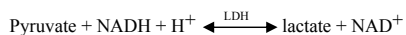
The reagents must be used only for *in vitro* diagnostic, by suitably qualified laboratory personnel, only for the intended purpose, under appropriate laboratory conditions.

INTRODUCTION

Lactate dehydrogenase (LDH, LD) is intracellular enzyme occurred in all tissues. LDH catalyzes the reversible conversion of lactate to pyruvate using 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 (H₄) through LD-5 (M₄). 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 Gessellschaft 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 mini	Liquick Cor-LDH 30	Liquick Cor-LDH 500
1-LDH	2 x 24 ml	5 x 24 ml	3 x 400 ml
2-LDH	1 x 12 ml	1 x 30 ml	1 x 300 ml

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.

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

Concentrations in the test

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

Liquick Cor-LDH

Warnings and notes

- Protect from direct sunlight and avoid contamination!
- Please refer to the MSDS for detailed information concerning safe storage and use of the product.
- 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

Applications for analyzers 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 μ l
Bring up to the temperature of determination. Then add:	
sample	20 μ l (temp. 25°C)
	or 10 μ 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:

λ	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 μ l
Bring up to the temperature of determination. Then add:	
sample	20 μ l (temp. 25°C)
	or 10 μ l (temp. 37°C)

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

2-LDH	250 μ l
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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:

λ	25°C	37°C
340 nm	10080	20000
334 nm	10275	20390
365 nm	18675	37060

REFERENCE VALUES ⁴

serum / plasma	37°C	
adults	225 – 450 U/l	3.75 – 7.50 μ kat/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 with each batch of samples the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173). 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

The following results 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 μ kat/l).
- Linearity:** up to 2000 U/l (33.3 μ kat/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) n = 20	Mean [U/l]	SD [U/l]	CV [%]
level 1	317.41	3.40	1.07
level 2	784.04	9.78	1.25
Reproducibility (day to day) n = 80	Mean [U/l]	SD [U/l]	CV [%]
level 1	312.47	3.26	1.04
level 2	782.43	7.43	0.95

Method comparison

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

y = 0.9227 x + 21.385 U/l;

R = 0.9952 (R – correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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