Lactat

Reagent for quantitative In-vitro-determination of lactate in blood, plasma and liquor

LAC 142

Order No. **LAC 142**
Content: 40 tests

Method
Enzymatic colorimetric test, LOD-PAP method[1)]
The determination can be carried out directly from blood. Blood will be immediately and completely haemolyzed by the reagent.

Sample material
Capillary or venous blood, plasma, liquor.
Serum cannot be used for the determination of lactate[1)].
In sport medicine only capillary blood (from hyperaemized ear lobe) is used. Blood has to be pipetted into the round cuvette immediately.
Stability* of the haemolyzed sample in the buffer solution:

at	+2°C	to	+8°C:	24 hours
at	+8°C	to	+20°C:	12 hours
at	+20°C	to	+30°C:	6 hours
at	+30°C	to	+40°C:	3 hours

* Increase in lactate concentration < 5 %

Obtain plasma by mixing approx. 2 mL of blood with 2 drops of fluoride/EDTA and centrifuging for approx. 5 min. at 3000 U/min within 2 hours.[2)] Stability of lactate in the decanted supernatant at +2 to + 8°C: 24h

Reagent
Contents / concentrations:
1. Starter reagent (caps in PE-bottle)
   Lactate oxidase (LOD) > 450 U/L, Peroxidase (POD) > 750 U/L, 4-Aminophenazone 0.23 mmol/L
2. Buffer solution (pre-portioned in round cuvettes)
   4-Chlorophenol 1.8 mmol/L, Sodium azide < 0.1 %, Triton X-100 < 1%, PIPES buffer 20 mmol/L

Safety information
The buffer solution (round cuvette) contains Sodium azide (<0.1 %) and Triton X-100. Do not swallow and avoid contact with skin and mucous membranes. If desired a safety data sheet will be provided.[3)]

Storage and shelf life
Reagents can be kept at a temperature between +2°C and +8°C until the expiry date indicated on the packaging.
Please take the screw caps out of the container just before the analysis and close the container immediately.

Measurement conditions
Measurement devices: Diaglobal Photometer

Meas. wavelength:	520nm
Temperature:	+5°C to +40°C
Meas. time	Ca. 3 - 4 min. (depends on temp.)

Measurement range
0.2 - 30 mmol/L (1.8 - 273 mg/dL)

Working instructions
The measurement can be performed as a single or serial measurement [with a balancing of the A(0) counts].

A. Single measurement

Pipette into round cuvette:	
	Analysis
Sample	10 µL
Mix thoroughly.	

- Select the <**LAC**> test
- Insert analysis cuvette (blank value)
- Screw the cap from PE-bottle onto the cuvette, dissolve the starter reagent by inverting several times
- Press [ON/ENTER]
- Insert analysis cuvette again
- Wait for result

B. Measurement of series (up to 20 samples)

Pipette into round cuvettes:	
	Analysis
Sample	10 µL
Mix thoroughly.	

- Select the <**LAC**> test
- Insert the analysis cuvettes one after another (blank values)
- Screw the caps from PE-bottle onto the cuvettes, dissolve the starter reagent by inverting several times
- Press [ON/ENTER]
- Insert the first analysis cuvette **immediately** again
- Wait for result
- Insert the other analysis cuvettes one after another in the same order as of the blank value measurement
- Results of the respective analysis cuvette can be read immediately

Quality assurance
For quality assurance we recommend the Diaglobal control set LAC QS.

Reference values[2)]

	mmol/L	mg/dL
Capillary blood	0.5 - 1.8	4.5 - 16.2
Plasma (venous)	< 2.2	< 19.8
Liquor	1.2 - 2.1	10.8 - 18.9

Summary
Lactate is the final product of the anaerobic glucose metabolism. It is increasingly produced when the rate of energy demand by somatic cells cannot be met by aerobic (involves oxygen) respiration due to an oxygen deficiency. Hence, high counts of lactate in resting indicate that several areas of the body are provided insufficiently with oxygen. Therefore, the lactate measurement is indispensable for numerous cases of emergency as, for example, in the case of shock, cardiovascular collapse, cardiac insufficiency, and metabolic acidoses. Furthermore, the determination of lactate is indicated when a bacterial meningitis and inflammatory cerebral diseases (here, apply sample material liquor) are existent.[2)]

The measurement of lactate has become notably important in sport medicine (performance diagnostics) and control of training cycles. Lactate is the most important indicator for the evaluation of the physical performance. On the basis of the lactate performance curve (representation of the lactate concentration depending on the degree of stress) the training condition may be evaluated and the optimal training pulse can be set up.[4)] A longer training above the anaerobic barrier (4 mmol/L) deteriorates the endurance performance ability. It is recommended to have lactate concentrations between 1.5 and 3.0 mmol/L for fitness and recreational sport.

It was only during the last quarter of the past century that the determination of lactate became important practically due to the development of a UV method which measures NADH, which is generated in a LDH catalysed reaction. The LOD-PAP method, which forms the basis of the Diaglobal test, is rested upon the enzymatic conversion of lactate by means of lactate oxidase (LOD) to pyruvate and the following conversion of the intermediary-generated H_2O_2 to a dye.[2)]

Measurement principle

$$\text{L-Lactate} + O_2 \xrightarrow{\text{Lactate oxidase}} \text{Pyruvate} + H_2O_2$$

$$H_2O_2 + \text{4-Chlorophenol} + \text{4-Aminophenazone} \xrightarrow{\text{Peroxidase}} \text{Quinonimine dye}$$

The concentration of the quinonimine dye is a measure for the lactate concentration in the blood and plasma respectively. It is measured photometrically at 520 nm.
The end point of the reaction is identified automatically by the device itself.

Performance parameters
Specificity / interferences
Neither lipaemia, bilirubin (up to 10 mg/dL), ascorbic acid in physiological concentrations (<30 mg/L) nor low and high haemoglobin levels will interfere with the determination. Pharmaceutical interferences: Low lactate counts due to Dopamine (10 mg/L), Levadopa (20 mg/L), and Methyldopa (20 mg/L). [5)] Interferences due to other pharmaceuticals are not known.

Inaccuracy
The reproducibility was checked using human and control samples.

In series [n = 20]	Average [mmol/L]	Standard deviation [mmol/L]	VK [%]
EDTA blood 1	1.58	0.04	2.8
EDTA blood 2	5.48	0.07	1.3
Control 1	3.95	0.07	1.7
Control 2	9.95	0.13	1.3
From day to day [n = 20]	**Average [mmol/L]**	**Standard deviation [mmol/L]**	**VK [%]**
Control 1	4.12	0.09	2.1
Control 2	10.1	0.19	1.9

Analytic sensitiveness
Lower detection limit: 0.2 mmol/L (1.8 mg/dL)

Comparison of methods
A comparison of the Diaglobal test LAC 142 (y) and two other commercially available tests (x) based on the UV and LOD-PAP method respectively resulted in the following correlation data according to the Passing/Bablok[6)] process:

a) LAC 142 / UV method:	b) LAC 142 / LOD-PAP:
Plasma	Haemolysate
y = 1,020x - 0,05	y = 1,016x + 0,03
r = 0,999	r = 0,998
n = 32	n = 46

Concentration range: 0.5 - 18 mmol/L

Information on disposal
Waste code number 180106:
Vials with reagent are considered hazardous waste. Do not allow reagent to reach surface water or sewage system.
Dispose of in accordance with official regulations.
Non-contaminated and completely empty packaging can be recycled.
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Bibliography
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3. http://www.diaglobal.de/de/service/downloads/index.html
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