Instructions for Use

For *in vitro* diagnostic use

LACTATO

Lactate

Anvisa 80115310042

ORDER INFORMATION

Cat No.	Kit size
1100075K	R1 3 x 20 mL + R2 1 x 15 mL
1100250K	R1 5 x 40 mL + R2 1 x 50 mL
1100075M	R1 3 x 20 mL + R2 1 x 15 mL
1100050MK	R1 1 x 40 mL + R2 1 x 10 mL
1100200R	R1 4 x 38,6 mL + R2 4 x 11,4 mL

INTENDED USE

Diagnostic reagent for quantitative *in vitro* determination of Lactate in plasma or cerebrospinal fluid (CSF) on photometric systems.

SUMMARY [1,2]

Lactate is the final product of the anaerobic glycolysis and serves as indicator for the oxygen status in cellular tissues. Increased lactate levels in blood occur in anoxia due to shock, congestive heart failure, intoxication and thiamine deficiency. Therefore, lactate is measured in intensive care medicine. As metabolic variable for the capability of the muscles, lactate determination is used in evaluation of the training status in athletes.

METHOD

Enzymatic UV test with lactate dehydrogenase (LDH)

PRINCIPLE

$$\text{L} - \text{Lactate} + \text{NAD}^+ \xleftrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH} + \text{H}^+$$

In the presence of NAD, lactate is converted to lactate dehydrogenase. This process releases NADH, which is measured at 340 nm. The absorbance of the NAD produced is proportional to the lactate concentration in the sample.

REAGENTS

Components and Concentrations

R1	Buffer	pH 9.0	500 mmol/L
	LDH		25 KU/L
R2	NAD		20 mmol/L

STORAGE AND STABILITY

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C, protected from light and if contamination is avoided. Do not freeze the reagents!

WARNINGS AND PRECAUTIONS

1. Reagent R1: Attention! Causes irritation in contact with the skin. It causes serious eye irritation. Wash hands and face after handling. Wear gloves, clothing, goggles and protective masks. In case of skin contact: wash thoroughly with soap and water. If skin irritation occurs, seek medical advice. If it has contact with the eyes: Rinse thoroughly with water for a few minutes. Remove contact lenses, if present, and continue rinsing. Contact a poison center or doctor if you feel unwell.
2. The reagent R1 contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. Reagent 1 contains biological material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
4. In very rare cases, samples of patients with gammopathy might give falsified results [6].
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only.

WASTE MANAGEMENT

Follow the requirements of the current guidelines about technical regulation for the management of healthcare service waste, as well as other equivalent biosafety practices.

REAGENT PREPARATION

Starting with Substrate

The reagents are ready to use.

Starting with Sample

Mix 4 parts of R1 with 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = monoreagent

The stability of the monoreagent is 14 days at a temperature of 2 to 8 ºC.

Do not use jaundiced or hemolyzed specimens at the start of the specimen.

MATERIALS REQUIRED, BUT NOT PROVIDED

1. NaCl solution 9 g/L.
2. General laboratory equipment.

SPECIMEN

Plasma or CSF (do not use serum)
Use glycolytic inhibitors e.g. fluoride/oxalate or fluoride/heparin as anticoagulants.

Stability in plasma[3]:	8 hours	at	20 - 25 ºC
	14 days	at	2 - 8 ºC

Discard contaminated specimens!

ASSAY PROCEDURE

Applications for automatic systems are available upon request

Wavelength	340nm
Optical path	1 cm
Temperature	37 ºC
Measurement	Against reagent blank

Starting with Substrate

	Blank	Sample or calibrator
Sample or calibrator	-	15 µL
Distilled water	15 µL	-
Reagent 1	1000 µL	1000 µL
Mix, incubate 5 min at 37 °C. Read absorbance A1, then add:		
Reagent 2	250 µL	250 µL
Mix, incubate 5 min at 37 °C. Read absorbance A2 within 30 min.		

$$\Delta A = (A_2 - A_1)_{\text{Sample/Calibrator}}$$

Starting with Sample (do not use icteric or hemolyzed samples)

	Blank	Sample or calibrator
Sample or calibrator	-	10 µL
Distilled water	10 µL	-
Monoreagent	1000 µL	1000 µL
Mix, incubate 5 min at 37 °C. Read absorbance within 30 min.		

CALCULATION

With calibrator

$$\boldsymbol{Lactate}\left[\frac{\boldsymbol{mg}}{\boldsymbol{dl}}\right] = \frac{\boldsymbol{\Delta A_{Sample}}}{\boldsymbol{\Delta A_{Calibrator}}} \times \boldsymbol{Calibrator\ Conc.}\,[\boldsymbol{mg/dL}]$$

With factor

From the absorbance readings calculate ΔA and multiply by the corresponding factor from the table below:

ΔA x factor = Lactate concentration [mg/dL]

	Starting with substrate	Starting with sample
340 nm	120.6	144.4

Conversion factor

Lactate [mg/dL] x 0.1109 = Lactate [mmol/L]

CALIBRATORS AND CONTROLS

For calibration in automated photometric systems, Kovalent Topkal U calibrator is recommended. Use Kovalent Topkon N and P for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

WARRANTY

These instructions for use should be read carefully before using the product and the information contained therein should be strictly adhered to. The reliability of the test results cannot be guaranteed if the instructions are not followed.

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PERFORMANCE CHARACTERISTICS

Measuring range

The test is designed to determine Lactate concentrations up to 120 mg/dL (13.3 mmol/L). When the values exceed this range, the samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity / Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, conjugated and unconjugated bilirubins up to 60 mg/dL, lipemia up to 2,000 mg/dL triglycerides, hemoglobin up to 1000 mg/dL, dopamine up to 10 mg/dL, L-dopamine up to 20 mg/L, methyldopamine up to 10 mg/L, and glycolic acid up to 1200 mg/L. For more information on interfering substances, see Young DS [4].

Sensitivity / Limit of Detection

The lowest detection limit is 1 mg/dL (0.1mmol/L).

Precision

Intra-assay precision n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	11.9	0.26	2.22
Sample 2	19.0	0.31	1.62
Sample 3	26.5	0.31	1.15

Inter-assay precision n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	12.0	0.23	1.91
Sample 2	19.0	0.28	1.45
Sample 3	26.7	0.31	1.16

Method comparison

Method comparison between Kovalent Lactato (y) and a commercial test available (x) using 117 samples demonstrated the following results:
y = 0.984 x – 0.742 mg/dL; r = 0.999.

REFERENCE VALUES [5]

	[mg/dL]	[mmol/L]
Plasma		
Venous	4.5 – 19.8	0.5 – 2.2
Arterial	4.5 – 14.4	0.5 – 1.6
CSF		
Adults	10 - 22	1.1 – 2.4
Newborn	10 – 60	1.1 – 6.7
3 – 10 days	10 - 40	1.1 – 4.4
> 10 days	10 - 25	1.1 – 2.8

Each laboratory should verify that reference values can be used in its own patient population and determine its own reference values, if necessary.

LITERATURE

1. David B. Sacks, M.B., Ch.B., F.A.C.P. Carbohydrates In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinicla Chemistry.
2. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 160-166.
3. Westgard JO, Lahmeyer BL, Birnbaum ML. Use of the Du pont “Automatic Clinical Analyzer” in Direct Determination of Lactic Acid in Plasma Stabilized with sodium Fluoride. Clin Chem 1972;18:1334-8.
4. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
5. Section I – General Clinical Tests In: Tietz NW, editor. Clinical Guide to Laboratory Tests. 3rd ed. Philadelphia: Saunders; 1995. p. 382-3.
6. Bakker AJ, Mucke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007; 45(9):1240-1243.

CONSUMER INFORMATION

Symbols used:

	Manufacturer
	Temperature limit
	In vitro diagnostic device
	Caution
	Operating instructions
	Recycling material
	Do not discard directly into the environment
	Batch code
	Date of manufacture
	Use by date
	Biological hazards
	Highly toxic
	Corrosive
	Harmful

Manufacturer:

Kovalent do Brasil Ltda.
Rua Cristóvão Sardinha, 110 – Jd. Bom Retiro
São Gonçalo – RJ – CEP 24722-414 - Brasil
www.grupokovalent.com.br
CNPJ: 04.842.199/0001-56

Customer service: sac@kovalent.com.br - (21) 3907-2534 / 0800 015 1414

Expiration date and Lot no.: See label