LDH DGKC

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Anvisa 80115310103

ORDER INFORMATION

Cat. No.	Kit size
2100075K	R1: 3 x 20 mL + R2: 1 x 15 mL
2100075M	R1: 3 x 20 mL + R2: 1 x 15 mL
2100200R	R1: 4 x 38,6 mL + R2: 4 x 11,4 mL
2100050MK	R1: 1 x 40 mL + R2: 1 x 10 mL

INTENDED USE

Diagnostic reagent for quantitative *in vitro* determination of Lactate Dehydrogenase (LDH) in serum or plasma on photometric systems.

SUMMARY [1,2]

Lactate dehydrogenase (LDH) is an enzyme, consisting of five different isoenzymes, which catalyze the interconversion of L-lactate and pyruvate. LDH is present in the cytoplasm of all human tissues with higher concentrations in liver, heart and skeletal muscle, and lower values in erythrocytes, pancreas, kidney and stomach. Increased LDH activities are found in a variety of pathological conditions such as myocardial infarction, cancer, diseases of liver, blood or muscle. However, because of the lack of organ specificity, determination of its isoenzymes or other enzymes such as alkaline phosphatase or ALAT/ASAT is necessary for differential diagnosis.

METHOD

Optimized test according to the German Society of Clinical Chemistry (DGKC) [3].

PRINCIPLE

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

REAGENTS

Components e Concentrations

R1	Potassium dihydrogen phospate		64 mmol/L
	Sodium pyruvate		<5 mmol/L
R2	Good's Buffer	pH 9.6	
	NADH		<5 mmol/L

STORAGE AND STABILITY

The 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!
Reagent 2 must be protected from light.

WARNINGS AND PRECAUTIONS

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. In very rare cases, samples of patients with gammopathy might give false results [7].
3. 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.
4. 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

Stability: 8 hours a 15 - 25 ºC
5 days a 2 - 8 ºC

The monoreagent must be protected from light.

MATERIALS REQUIRED, BUT NOT PROVIDED

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

SPECIMEN

Serum, heparin plasma or EDTA-plasma

Stability [4]: 4 days a 20 - 25 ºC
6 weeks a 4 - 8 ºC

Discard contaminated specimens.

ASSAY PROCEDURE

Applications for automatic systems are available upon request or on our website: www.kovalent.com.br

Wavelength	340 nm, Hg 365 nm, Hg 334 nm
Optical path	1 cm
Temperature	25 ºC / 30 ºC / 37 ºC
Measurement	Against air

Starting with Substrate

Temperature	25 ºC or 30 ºC	37 ºC
Sample or calibrator	20 µL	10µL
Reagent 1	1000 µL	1000 µL
Mix, incubate for approximately 1 – 5 minutes, then add:		
Reagent 2	250 µL	250 µL
Mix, read the absorbance after 1 min and start the stopwatch. Read the absorbance again after 1, 2 and 3 min.		

Starting with Sample

Temperature	25 ºC or 30 ºC	37ºC
Sample	20 µL	10µL
Monoreagent	1000 µL	1000 µL
Mix, read the absorbance after 1 min and start the stopwatch. Read the absorbance again after 1, 2 and 3 min.		

CALCULATION

With factor

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

ΔA/min x factor = LDH Activity [U/L]

Starting with Substrate	25 ºC or 30 ºC	37 ºC
340 nm	10080	20000
334 nm	10275	20390
365 nm	18675	37060
Starting with Sample	25 ºC or 30 ºC	37 ºC
340 nm	8095	16030
334 nm	8250	16345
365 nm	15000	29705

With calibrator

$$\text{LDH [U/L]} = \frac{\Delta A/\text{min}_{\text{Sample}}}{\Delta A_{\text{Cal.}}} \times \text{Conc. Cal. [U/L]}$$

Conversion factor

LDH [U/L] x 0.0167 = LDH [µkat/L]

CALIBRATORS AND CONTROLS

For calibration in automated photometric systems, Kovalent Topkal U calibrator is recommended. This method has been standardized against the original IFCC formulation. 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.

PERFORMANCE CHARACTERISTICS

Measuring range

In automated systems the test is suitable for the determination of LDH concentrations within a range of 5 - 1200 U/L.
In the case of manual procedure, the test is suitable for LDH activities, which correspond to a maximum ΔA/min of 0.15 at 340 and 334 nm or 0.08 at 365nm.
If these values are exceeded, the samples should be diluted 1 + 10 with NaCl solution (9 g/L) and the results multiplied by 11.

Specificity / Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL and lipemia up to 2000 mg/dL of triglycerides. Hemolysis interferes because LDH is released by erythrocytes. For more information on interfering substances, see Young DS [5].

Sensitivity / Limit of Detection

The lowest detection limit is 5 U/L.

Precision

Within run n = 10	Mean [U/L]	SD [U/L]	CV [%]
Normal control	235.8 246.4	2.80 2.41	1.19 0.98
Pathological control	514.8 451.6	3.29 2.12	0.64 0.47

Between day n = 15	Mean [U/L]	SD [U/L]	CV [%]
Normal control	233.3 247.3	3.10 3.59	1.33 1.45
Pathological control	514.0 448.3	6.02 4.56	1.17 1.02

Method comparison

Method comparison between Kovalent LDH (y) and a commercially available test (x) using 30 samples demonstrated the following results:
$y = 0.9805x + 9.7218$; $R^2 = 0.9818$.

REFERENCE VALUES [6]

	25 ºC	30 ºC	37 ºC	
Adults	< 240	< 346	< 480	[U/L]
	< 4	< 5.77	< 8	[µkat/L]

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

LITERATURE

1. Thomas L. Clinical laboratory diagnostics. 1º ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998.p.89-94.
2. Moss DW, Henderson AR. Clinical enzymology In: Burtis CA, Ashwood ER, editores. Tietz Textbook of Clinical Chemistry. 3º ed. Filadélfia: W.B Saunders Company; 1999.617-721.
3. Deutsche Gesellschaft für klinische Chemie. Empfehlungen der deutschen Gesellschaft für Klinische Chemie (DGKC). Standardisierung von Methoden zur Bestimmung von Enzymaktivitäten in biologischen Flüssigkeiten. (Recommendation of the German Society of Clinical Chemistry. Standardization of methods for measurement of enzymatic activities in biological fluids.) Z Klin Chem Klin Biochem 1972; 10:182- 92.
4. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1º ed. Darmstadt: GIT Verlag; 2001; p. 36-7.
5. 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.
6. Fischbach F, Zawta B. Age-dependent reference limits of several enzymes in plasma at different measuring temperatures. Klin Lab 1992; 38:555-61.
7. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 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.
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São Gonçalo – RJ – CEP 24722-414 - Brasil
www.kovalent.com.br
CNPJ: 04.842.199/0001-56

Kit sizes variations on demand:

Anvisa No.	Kit size
80115310103	R1: 2 x 50 mL + R2: 2 x 12,5 mL
80115310103	R1: 2 x 40 mL + R2: 2 x 10 mL
80115310103	R1: 3 x 40 mL + R2: 3 x 10 mL
80115310103	R1: 4 x 40 mL + R2: 4 x 10 mL

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Expiration date and Lot no.: See label