N BIO - LDH

(DGKC method)

KIT NAME	KIT SIZE	CAT. NO
N BIO - LDH	2 x 25 ml	DLDH02025M
N BIO - LDH	2 x 50 ml	DLDH02050M

INTRODUCTION

LDH is mainly found in many body tissues particularly heart, liver, skeletal muscle, kidney and RBC's. LDH is found in the form of isoenzymes based on their electrophoretic mobility with each isoenzyme being primarily from different organs. Increased levels are found in myocardial infarction, pulmonary diseases, hemolytic anemias, renal diseases, and muscular dystrophy.

METHOD PRINCIPLE

Lactate Dehydrogenase catalyzes the reduction of pyruvate with NADH to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the LDH activity in the sample.

Pyruvate + NADH + H+ ------> lactate + NAD+

The rate of absorbance changing at I=340 nm is directly proportional to lactate dehydrogenase activity

KIT CONTENTS

Reagent Name	DLDH02025M	DLDH02050M
R1 LDH Reagent	2 x 20 ml	2 x 40 ml
R2 LDH Reagent	2 x 5 ml	2 x 10 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 2 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 R1-LDH and R2-LDH reagents or with use of working reagent. For working reagent preparation mix gently 4 parts of R1-LDH with 1 part of R2-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

Tris buffer (pH 6.8)	100 mmol/l
EDTA	0.07 gm/l
sodium pyruvate	1.20 mmol/l
NADH	0.28 mmol/l
sodium chloride	160 mmol/l

WARNINGS AND NOTES

- · Product for in vitro diagnostic use only.
- The reagents contain 0.09% sodium azide 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 λ=340 nm, cuvette l=1 cm, at temp. 25°C).

ADDITIONAL EOUIPMENT

-Automatic analyzer or photometer able to read at 340 nm (H 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, but it is recommended to perform the assay with freshly collected samples.

PROCEDURE

These reagents may be used both for manual assay and in several automatic analysers. Applications for them are available on request.

Wavelength 340 nm Temperature 37°C Cuvette 1 cm

Pipette into the cuvette:

Reagent	Test (T)	
R1 LDH reagent	800 μl	
R2 LDH reagent	200 μ1	
Bring to assay temperature, then add		
Sample	20 μl	

Mix and incubate at adequate temperature. After about 1 min. read the absorbance against air or water. Repeat the reading after exactly 30, 60 & 90 Seconds interval. Calculate the mean absorbance change per minute ($\triangle A/min$.).

CALCULATION

LDH activity $[U/l] = \triangle A/min. \times 9500$

REFERENCE VALUES

225 - 450 U/L

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

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

PERFORMANCE CHARACTERISTICS

- Sensitivity / Limit of Quantitation: 25 U/L.
- Linearity: up to 2000 U/L. If LDH activity in tested sample 2000 U/Ldilute the sample with 0.9% NaCl in the ratio of 1 to 9 and repeat the assay, multiply the result by 10.
- Specificity / Interferences
 Bilirubin up to 20 mg/dl, haemoglobin up to 12.5 g/dl, ascorbate up to 62 mg/l and triglycerides up to 500 mg/dl do not interfere with the test.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

- 1. Thomas L. clinical laboratory diagnostics 1st ed. Frankfurt: TH books verlagsgesellschatt; 1998:89-44
- NCCLS document "Evaluation of precision performance of clinical chemistry devices, 2nd ed. (1992).
- Moss D.W., Henderson, A.R Clinical Enzymology In; Burtis C.A. ashwood E.R editors, Tietz Textbook of clinical chemistry . 3rd ed. Philadelphia. W.B Saunders Company; 1999:617-721.

SYSTEM PARAMETERS

Method	Kinetic
Wavelength	340 nm
Zero Setting	Distilled Water
Temperature Setting	37° C
Incubation Temperature	37° C
Incubation Time	
Delay Time	60 secs
Read Time	120 secs
No. of Reading	4
Interval Time	30 secs
Sample Volume	0.02 ml (20 ul)
Reagent Volume	1.0 ml (1000 ul)
Standard Concentration	
Units	U/L
Factor	9500
Reaction Slope	Decreasing
Linearity	2000 U/L





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