MEDICA

℞Only IVD CE

REF 10215-4 4 x 29 mL / 8 mL

LACTATE DEHYDROGENASE (LDH)

Each wedge contains a usable volume of 29 mL of R1 reagent and 8 mL of R2 reagent.

INTENDED USE

The EasyRA LDH reagent is intended for the quantitative determination of Lactate Dehydrogenase (LDH) activity in human serum and plasma (using lithium heparin as anticoagulant), using the MEDICA EasyRA® clinical chemistry analyzer.
For *in vitro* diagnostic use. For professional use only.

SUMMARY AND EXPLANATION

Lactate Dehydrogenase is a combination of isoenzymes that are elevated by myocardial infarction, liver and renal disease, megaloblastic anemias and advanced muscular dystrophy.[1] A case for acute myocardial infarction can be made based on the combined analysis of LDH and Creatine Kinase levels.[2]

PRINCIPLE OF THE PROCEDURE

The enzymatic assay procedure is based on the work of Wacker[3] and includes the improvements by Buhl and Jackson.[4] LDH catalyzes the oxidation of lactate to pyruvate and the subsequent reduction of NAD to NADH as follows:

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

The rate of the formation of NADH (measured by its absorbance at 340 nm) is proportional to the Lactate Dehydrogenase activity in the sample.

REAGENTS

LDH Buffer Reagent (R1):

L-Lactate	65 mmol/L
N-Methyl-D-Glucamine pH 8.4	420 mmol/L

LDH Substrate Reagent (R2):

NAD	50 mmol/L

PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A3).
2. The reagents contain less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2 – 8°C. The reagent is stable on the EasyRA clinical chemistry analyzer for the number of days programmed on the RFID chip on the reagent wedge. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values.

SPECIMEN COLLECTION AND STORAGE / STABILITY

Clear non-hemolyzed serum or plasma should be used. Lithium heparin coated tubes may be used for plasma collection. Centrifuge and remove the serum as soon as possible after collection because hemolysis causes erythrocytes to release large amounts of LDH. LDH is stable in serum or plasma for 3 days at 2 – 8°C.[5] Frozen samples show decreased activity in LDH values. Protect from light.

PROCEDURE

Materials Provided

Medica LDH Reagent Wedge, REF 10215-4

Additional Materials Required

Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC® Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use
The reagent is ready to use as supplied. Remove the cap and place the reagent in the EasyRA clinical chemistry analyzer reagent tray located in the reagent area. The on-board stability (60 days maximum) is programmed on the RFID chip on the reagent wedge.

Note: Check inside the necks of the wedge for foam after removing the caps and placing the wedge on the EasyRA clinical chemistry analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2.

Calibration

Not applicable.

Quality Control
It is recommended that two levels of human serum-based controls (normal and abnormal) be run with the assay daily whenever patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results
After completion of the assay, the EasyRA clinical chemistry analyzer calculates the LDH concentration from the change in absorbance per minute, sample volume, total reaction volume, pathlength (cm) of 0.6 and molar absorptivity of 6.22.

$$\text{LDH (U/L)} = (\Delta A/\text{Min}) \times \frac{(\text{Total Volume}(\mu l) \times 1000)}{(\text{Molar absorptivity} \times \text{Pathlength(cm)} \times \text{Sample Volume}(\mu l))}$$

A unit per liter (U/L) of LDH activity is the amount of enzyme, which produces one μmol/L of NADH per minute.

EXPECTED VALUES[6]
The reference range for LDH in serum and plasma is as follows:

Male: 80 – 285 U/L (37°C)
Female: 103 – 227 U/L (37°C)

These values are guidelines. It is recommended that each laboratory establish its own range of expected values since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g. if sample is above assay range)
If the Absorbance change per Minute (ΔA/Min) is greater than 0.0902, which corresponds to approximately 800 U/L, it will be flagged with “SD” (substrate depletion) by the analyzer. If the “Re-run” icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the LDH test to 1600 U/L.

PERFORMANCE CHARACTERISTICS[7]

Reportable Range
The reportable range is 5 to 800 U/L. Extended range is 5 to 1600 U/L when half of the sample is used (1:1 dilution).

Inaccuracy / Correlation (CLSI, EP09-A2)
The following table lists the data obtained in a comparison of the Medica reagent for LDH (y) on the EasyRA clinical chemistry analyzer to the performance of a similar LDH reagent (x) on the Roche COBAS MIRA* analyzer. The data shown below represents single determinations obtained on the EasyRA clinical chemistry analyzer vs. the average of two replicate values obtained on the COBAS MIRA analyzer.

Number of samples	59	Range of Samples	5 to 779 U/L
Slope	0.99	y Intercept	8.4
Correlation Coefficient	0.9980	Regression Equation	Y = 0.99*X + 8.4

The following table lists the data obtained in a comparison of matched serum (x) and plasma (y) samples using the Medica reagent for LDH on the EasyRA clinical chemistry analyzer. The data below represents a single plasma determination vs. the average of two replicate serum values.

Number of samples	47	Range of Samples	10 to 696 U/L
Slope	0.9975	y Intercept	2.825
Correlation Coefficient	0.9949	Regression Equation	Y = 0.9975*X + 2.825

**Cobas Mira is a registered trademark of Roche Diagnostics Operations, INC., Indianapolis, IN.*

Imprecision (CLSI, EP05-A2)
Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

QC Level U/L	Within Run SD U/L	Within Run CV %
331.5	4.0	1.2
204.5	3.3	1.6
99.5	1.3	1.3

Total Imprecision:

QC Level U/L	Total Imprecision SD U/L	Total Imprecision CV %
331.5	7.3	2.2
204.5	4.8	2.4
99.5	1.9	1.9

Linearity (CLSI, EP06)

Linear from 5 to 800 U/L, based on the linear regression equation Y = 1.017*X + 14.48.

Interfering Substances (CLSI, EP07)

Less than 10% interference was classified as “no significant interference.”
There is significant interference from hemoglobin. Do not use hemolyzed samples.
No significant interference was found in levels up to 20 mg/dL of bilirubin.
No significant interference was found in levels of up to 2000 mg/dL of triglycerides (using Intralipid*).
No significant interference was found in levels of up to 30 mg/dL of ascorbic acid.

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC.*

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[8, 9]

REFERENCES

1 Kachmar JF, Moss DW in Tietz Fundamentals of Clinical Chemistry, 2nd Ed.,NW Tietz, editor (W.B Saunders, Eds. Philadelphia USA) 1976: 652.
2 Roe CR et al., J Lab Clin Med 80:557 (1972).
3 Wacker WEC et al., New England J Med 255: 449(1956)
4 Buhl SN, Jackson KY, Clin. Chem. 24:828 (1978).
5 Guder WG, Zawta B et el. The Quality of Diagnostic Samples. 3rd edition; 2010: p. 52-3.
6 Henry, RJ, Carman DC, and Winkelman JW. *Clinical Chemistry. Principles and Techniques*. Haggerstown, MD: Harper and Row. pp 819-831 (1974).
7 Data on file at Medica.
8 Young DS. *Effects of Drugs on Clinical Laboratory Tests* 4th ed. Washington, DC: AACC Press; 1995.
9 Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press; 1997.

EASYRA ASSAY PARAMETERS (LDH)

Wavelength (nm)	340
Reaction type	Enzyme (0)
Reaction direction	Increase
Reagent blank	No
Sample blank	No
Max. first interval Abs. change	0.055
Reaction time	4.4 min
Calibration interval (maximum)	N/A
Reagent on-board stability	60 days

Serum/Plasma

Sample volume (µl)	7.0
Diluent volume (µl)	20
Reagent volume (R1) (µl)	132
Reagent volume (R2) (µl)	34
Decimal places (default)	0
Units (default values)	U/L
Dilution factor	1:1 (to extend measuring range)
Linearity	5 to 800 U/L