LACTATE DEHYDROGENASE (LD) (LDH) REAGENT KIT C144-0B

Contents	Product No.	Package
LACTATE DEHYDROGENASE REAGENT KIT	**C144-0B**	
Substrate Reagent (R1)	C144-03	6 x 46 mL
NAD Reagent (R2)	C144-04	6 x 4 mL

REAGENT PREPARATION for *INDIVIDUAL* R1 and R2 use

The Catachem Lactate Dehydrogenase reagents are both liquid, ready to use reagents.
No preparation is needed when used as a two-reagent system.

REAGENT PREPARATION for *SINGLE* WORKING REAGENT USE

Prepare the Catachem Lactate Dehydrogenase as a Single Working Reagent by pouring one bottle of the NAD Reagent (R2) into one bottle of the Substrate Reagent (R1).
Mix by inverting several times.

REAGENT STORAGE AND STABILITY

Store unopened reagents at 2-8°C.
When stored as directed, the reagents are stable until the expiration date stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
Email: catachem@catacheminc.com
Contact Form: www.catacheminc.com
Call: 203-262-0330

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LACTATE DEHYDROGENASE (LD) (LDH)
C144-0B
MANUAL/AUTOMATED APPLICATION

Intended Use
For **IN VITRO** quantitative determination of Lactate Dehydrogenase in serum.

Clinical Significance
Measurements of LD activity are primarily used for diagnosing myocardial infarctions, myocarditis, cardiac failure with hepatic congestion, liver disease, pernicious anemia, megaloblastic anemia and renal disease, as well as for monitoring the causes and treatments. [1]

Method History
The Catachem Lactate Dehydrogenase (LD) method for manual or automated chemistry is based upon the work of Wacker, et al [2] who described a method for the measurement of LD utilizing lactate as substrate and nicotinamide-adenine dinucleotide as cofactor and as the reaction indicator.

Method Principle
Lactate Dehydrogenase (EC 1.1.1.27; L-lactate: NAD oxido reductase) is an enzyme which catalyzes the oxidation of L-lactate to pyruvate with the mediation of NAD as a hydrogen acceptor. The reaction is reversible and although it favors the conversion of pyruvate to lactate [2] the Catachem method uses the less favored reaction. The rate of increase in absorbance of the reaction mixture due to the formation of NADH is proportional to the LD concentration present in the serum sample. The reaction scheme illustrates the reaction that occurs in this method.

$$\text{L-lactate} + \text{NAD} \xrightarrow{\text{LD}} \text{Pyruvate} + \text{NADH} + \text{H}^+$$

Reagent Content
When reconstituted according to the directions, the concentrations of the active ingredients will be approximately as follows:

LD Substrate
Each vial contains:

L-lactic acid lithium salt	50 mmol/L
Buffer	
Nonreactive ingredients and stabilizer	

LD NAD
Each vial contains:

Nicotinamide Adenine Dinucleotide (NAD)	103 mmol/L
Nonreactive ingredients and stabilizer	

Preparation of Working Reagents
If a single working reagent is required, prepare Catachem LD Working Reagent by adding the contents of the appropriate vial containing LD NAD into the appropriate bottle containing the LD substrate. Mix and label this reagent “LD Working Reagent”. (See previous instructions for more details.) The reagent is usually used as a dual R1/R2 reagent on an automated analyzer.

Reagent Storage And Stability
Store the LD reagents (liquid) at 2-8˚C. When stored as directed, these reagents are stable until the expiration date stated on the label. After opening, each reagent is stable for 30 days when stored at 2-8 ˚C and capped when not in active use. The single working reagent when prepared and stored capped when not in active use at 2-8 ˚C is stable for five days. If stored at room temperature, the working reagent is stable for eight hours. The Catachem LD Reagents have been tested to reflect shipping conditions and are stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures up to 40°C for up to one week.

Specimen Collection And Preparation
The use of clear, non-hemolyzed serum that has been separated from the clot as soon after collection as possible, is recommended.

Precaution
Avoid contact of specimen with skin. Should contact occur, wash affected area with plenty of water. **DO NOT PIPETTE SPECIMENS BY MOUTH.**

Interfering Substances
A comprehensive discussion has been reported on the effect of interfering substances on various LD methods. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al. [3]

Expected Values
The normal human range for this assay as performed below is 120 U/L to 230 U/L. These values serve as suggested reference points only. For veterinary samples, ranges will be dependent on the species under test. It is recommended that each laboratory establish the normal ranges for the species under study and for the geographic area in which the laboratory is located.

Procedure
Important: Read the entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer	
Match cuvettes	1 cm light path
Timer	To time incubation time
Pipette	1.0 ml for reagent
Pipette	0.050 ml for sample
Pipette or graduated cylinder	25 or 50 ml for reagent

Materials Provided
LD Reagents

Quality Control
To ensure optimal performance of these reagents and this procedure, we recommend systematic calibration using Catachem’s Catacal (C1200-10). Assay performance should be monitored by running normal/abnormal controls concomitantly with samples. Catachem has optimized this assay using Catatrol Level I (C1200-11) and Catatrol Level II (C1200-12) and recommends their use for daily QC.

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CATACHEM

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Calibration
This method can be calibrated as noted above or can be used without specific calibration in which the extinction configuration and assay parameters are used to determin LD activitity. This method is outlined below.

Analytical Parameters

Wavelength	340 nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	zero-order rate, direct
Reaction Time	2.0 min. 37˚C
Reagent Volume	1.0 ml
Sample Volume	0.05 ml
Total Volume	1.05 ml
Sample-to-Reagent Ratio	1:21

Assay Procedure
1. Into separate cuvettes, pipette 1 ml Working Reagent.
2. Pipette 0.05 ml of "Control" or "Sample" into their respective cuvettes. Mix all cuvettes well.
3. Incubate all cuvettes for exactly 30 seconds. Read the "Control" and each "Sample" absorbance at 340nm at 90 and 150 seconds to determine the OD change per minute. (Δ A/min)

Calculations And Results

$$\text{LD U/L} = \frac{\Delta \text{ A/min} \times 1.05 \text{ ml} \times 1000}{6.22 \times 1 \text{ cm light path} \times 0.05 \text{ ml}}$$

LD U/L = A/min x 3376

A/min	= Change in absorbance per minute
1.05 ml	= Total assay volume in ml
1000	= Converts u/ml to u/L
6.22	= Absorbance coefficient of NADH at 340nm
Light Path	= 1 cm
0.05 ml	= Sample volume
3376	= Factor derived from constants in the equation

Example:
Sample A/min = 0.03
LD U/L = 0.03 x 3376 = 101 u/L

Method Performance Characteristics
***Sensitivity*:** The sensitivity of this method is 0.0002 absorbance units per U/L.
***Linear Range*:** No significant nonlinearity over the range of 0-600 U/L.

Precision Study

LD	TOTAL		WITHIN-RUN	
MEAN	SD	CV	SD	CV
U/L	U/L	%	U/L	%
61	0.90	1.40	1.20	1.80
391	3.40	1.20	2.90	0.90
546	5.70	1.00	3.70	0.60

Correlation
A comparison of the Catachem LD method using an automated analyzer was carried out against a reference LD procedure based upon the same principle as the Catachem LD method resulted in the following regression statistics:

Range	=	100 - 431 U/L
N	=	123
Y	=	0.999x + 0.08
r	=	0.999
Sy.x	=	3.7

References
1 Fundamentals of Clinical Chemistry. Edited by Norbert W. Tietz. W.B. Saunders Co. pp 652-660 (1967).
2. Wacher Wec, Ulmer Vallee. Metaloenzymes and myocardial infarction. New England J Med 225, 449 (1956).
3. Young, Pestaner, Gibberman. Effects of drugs on clinical laboratory tests. Clin Chem 21 No 5 (1975).

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