

# LDH (Liquid)

## 1.0 INTENDED USE

This reagent is intended for the quantitative determination of lactate dehydrogenase (LDH) in serum.

## 2.0 BACKGROUND

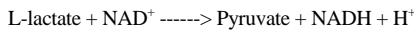
### 2.1 METHOD AND HISTORY

Wroblewski and LaDue (10.2) published the first UV kinetic method for the determination of LD activity in serum in 1955. Their method was based on the classic Kubowitz and Ott (10.3) assay (1943) utilizing the pyruvate to lactate reaction. In 1956, Wacker et al (10.4) described a procedure that followed a lactate to pyruvate reaction. The lactate to pyruvate reaction became the preferred reaction (10.5), even though the slower of the two, because of a wider linear range (10.6) and no pre-incubation requirement (10.7). The present method follows the forward reaction and has been optimized for greater sensitivity and linearity as outlined by Gay et al. (10.8)

### 2.2 TEST PRINCIPLE

Lactate dehydrogenase catalyzes the conversion of L-Lactate to pyruvate in the presence of NAD. The rate of enzymatic activity of LD is proportional to the rate of production of NADH at 340 nm.

#### LDH



Lactate dehydrogenase catalyzes the oxidation of lactate to pyruvate with simultaneous reduction of NAD to NADH. The rate of NAD reduction can be measured as an increase in absorbance at 340nm. This rate is directly proportional to LD activity in serum.

### 2.3 CLINICAL SIGNIFICANCE (10.1)

Increased levels of LD are associated with myocardial infarction. Levels reach a maximum approximately 48 hours after the onset of pain and persist about ten days. The degree of elevation is of value in assessing the extent of damage and in developing a prognosis. LD elevations are also observed in liver disease, pernicious anemia, in some cases of renal disease, and in some cases of skeletal muscle trauma. (10.1)

## 3.0 SPECIMEN COLLECTION AND HANDLING

### 3.1 PATIENT PREPARATION

No special patient preparation is required.

### 3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen. Serum with any visible hemolysis should not be used. Due to large amounts of LDH released from the erythrocytes, serum should be separated from the clot as soon as possible. Use a standard venipuncture tube to draw patient sample. The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-50 µl. Call Biotron's technical service department at 1-800-595 8766 for the recommended sample volume for your analyzer. Record the patient's name, date and time of sample collection and preparation.

### 3.3 SPECIMEN STORAGE

Serum samples can be stored at 2°-8°C for up to 3 days with no loss of activity. (10.4) The liver enzyme is particularly labile and is destroyed if frozen and thawed.

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

## 4.0 MATERIALS (1X120,1X30 ml)

Reagents necessary for the determination of LDH are included in the kit.

### 4.1 REAGENT

LDH working reagents contains:

NAD	> 7.5 mM
L-lactate	> 55 mM
sodium azide	0.1%
buffer, non-reactive stabilizers and fillers	

## 4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact. This reagent contains sodium azide (0.1%) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large amounts of water.

## 4.3 REAGENT PREPARATION

The working reagent is prepared by combining 4 parts of R1 (buffer) to 1 part of R2 (coenzyme).

## 4.4 REAGENT STORAGE AND STABILITY

All the reagents included in the kits are stable at 2-8° C (refrigerated) until the expiration date stated on the labels. The working reagent is stable at 2-8° C (refrigerated) for 21 days.

## 4.5 ADDITIONAL MATERIALS REQUIRED

- 4.5.1 Spectrophotometer or colorimeter capable of reading absorbance at 340 nm.
- 4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 340 nm.
- 4.5.3 Test tube and pipettes.
- 4.5.4 Timer with one minute increments.
- 4.5.5 Constant temperature source which can be adjusted to 37° C.
- 4.5.6 Normal and abnormal controls for quality control.

## 5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument. Application procedures for use on automated analyzers are available. Contact Biotron's Technical Service Department for specific information.

### 5.1 PROCEDURE CONDITIONS

Wavelength	340 nm
Temperature	37° C
Pathlength	1.0 cm
Mode	kinetic
Lag time	30 seconds
Sample to reagent ratio	1:20

### 5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 340 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

### 5.3 CALIBRATION

No reagent calibration is necessary as this procedure is standardized based on the millimolar absorptivity of NADH which is taken as 6.22 at 340 nm under the test conditions described.

### 5.4 PROCEDURE

- 5.4.1 Prepare the required volume of working reagent. (See 4.3 Reagent Preparation Section.)
- 5.4.2 Into separate test tubes pipette 50 µl of serum to be assayed.
- 5.4.3 Add 1.0 ml of working reagent, mix, and incubate for 30 seconds at 37° C.
- 5.4.4 Record the increase in absorbance at 340 nm at one minute intervals until the absorbance change is constant.

### 5.5 CALCULATION AND RESULTS

Lactate dehydrogenase U/L =  
$$\frac{\Delta A/\text{min} \times \text{assay volume (ml)} \times 1000}{6.22 \times \text{light path (cm)} \times \text{sample volume (ml)}} = \Delta A/\text{min} \times 3376$$
  
$$\Delta A/\text{min} = \text{change in absorbance per minute}$$
  
Assay volume = 1.050 (ml)  
1000 = converts U/ml to U/L  
6.22 = absorbance coefficient of NADH at 340 nm  
Light path = length of light path (usually 1 cm)  
Sample volume = 0.050 (ml)  
3376 = factor derived from constants in the equation  
Example: Lactate dehydrogenase U/L =  
$$0.05 \times 1.050 \times 1000$$
  
----- = 0.05 X 3376 = 169 U/L with  $\Delta A/\text{min} = 0.05$   
$$6.22 \times 1 \times 0.050$$

**6.0 INTERPRETATION OF RESULTS**

**6.1 EXPECTED VALUES (10.5)**

The range of expected values is:

Male: 90-221 U/L (37° C)

Female: 89-187 U/L (37° C)

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

**6.2 MEDICAL ALERT VALUES (10.7)**

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

**6.3 LIMITATIONS OF PROCEDURE**

Oxalates, oxamates, and EDTA inhibit LDH. Young (10.6) gives a list of drugs and other substances that interfere with the determination of LDH activity.

**7.0 QUALITY CONTROL**

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

**8.0 CALIBRATION PROCEDURES**

No routine reagent calibration is necessary as this procedure is standardized based on the millimolar absorptivity of NADH which is taken as 6.22 at 340 nm under the test conditions described.

**9.0 PERFORMANCE CHARACTERISTICS**

**9.1 PRECISION**

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run

Mean (U/L)	SD (U/L)	CV (%)
183	2.59	1.4
441	3.76	0.9

Between-Run

Mean (U/L)	SD (U/L)	CV (%)
189	5.53	2.9
443	6.33	1.4

**9.2 CORRELATION**

A correlation study was done this method and a comparative UV LDH method. The samples range between 80 and 540U/L.

Number of Samples	Regression Equation y=Biotron, x=Comparative	Correlation Coefficient
103	y = 1.00 x + 7.4	0.994

**9.3 LINEARITY**

This procedure is linear through 1000 U/L, beyond which the specimen should be diluted with an equal volume of deionized water. Reassay the specimen and multiply the results by 2.

**9.4 SENSITIVITY**

An absorbance change of 0.001ΔA/min corresponds to approximately 3.4 U/L LDH activity.

**10.0 REFERENCES**

10.1 Tietz, N.W., editor, Fundamentals of Clinical Chemistry, 3<sup>rd</sup> Ed., W.B. Saunders Co., 391(1987).  
 10.2 Wroblewski, F., La Due, J.S., Proc. Soc. Exp. Biol. Med. 90:210(1955).  
 10.3 Kubowitz, F., Ott, P., Biochem. 314:94(1943).  
 10.4 Wacker, W.E.C., et al, N. Engl. J. Med. 255:449(1956).  
 10.5 Henry, R.J. et al, Clinical Chemistry; Principles and Technics, 2<sup>nd</sup> Ed., Hagerstown (MD) Harper & Row, pp.819-831,(1974).  
 10.6 Amador, E., et al, Clin. Chem. 9:391(1963).  
 10.7 Buhl, S.N., et al, Clin. Chem. 23:1289(1977).  
 10.8 Gay, R.J., McComb, R.B., Bowers, G.N. Clinical Chemistry, 14:740(1968).  
 10.9 Teitz, N.W., Fundamentals of Clinical Chemistry, 2<sup>nd</sup> Ed., W.B. Saunders Co., 657,(1976).  
 10.10 Kreutzer, H.H., et al, Clin. Chim. Acta 9:64(1964).  
 10.11 NCCLS Document M29-T2, 2<sup>nd</sup> Ed.(1991).  
 10.12 Young, D.S., et al, Clin. Chem., 21:1D (1975).