Pointe Lactate Dehydrogenase Reagent Set

HORIBA Instruments Incorporated
5449 Research Drive, Canton, MI 48188
Phone: 734-487-8300; (800) 445-9853

Intended Use
For the *in vitro* quantitative kinetic determination of lactate dehydrogenase activity in serum using the Yumizen C560 analyzer. **Rx Only**.

Clinical Significance
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.[1]

Method History
Wroblewski and Ladue[2] published the first UV kinetic method for the determination of LDH activity in serum in 1955. Their method was based on the classic Kubowitz and Ott[3] assay (1943) utilizing the pyruvate to lactate reaction. In 1956, Wacker et al[4] described a procedure that followed a lactate to pyruvate reaction. The lactate to pyruvate reaction became the preferred reaction[5], even though the slower of the two, because of a wider linear range[6] and no pre-incubation requirement[7]. The present method follows the forward reaction and has been optimized for greater sensitivity and linearity as outlined by Gay et al.[8]

Principle
$$\text{L-Lactate} + \text{NAD+} \xrightarrow{\text{LD}} \text{Pyruvate} + \text{NADH} + \text{H+}$$

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.

Reagent Composition
After combining R1 and R2 the reagent contains: NAD 5.8 mM, L-Lactate 55 mM, Buffer pH 8.95. Non-reactive stabilizers and sodium azide (0.1%) as preservative.

Reagent Preparation
Reagents are supplied as ready to use liquids.

Reagent Storage and Stability
Reagents are stable until stated expiration if stored as directed. Protect from light. Avoid microbial contamination. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

Precautions and Hazards
1. This reagent is for *in vitro* diagnostic use only.
2. All specimens and controls should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories," 2nd ed., 1988, HHS Publication No. (CDC) 88-8395.
3. The reagents contain sodium azide (0.1%) as a preservative. Do not ingest. Avoid skin and eye contact. Sodium azide may react with lead and copper plumbing fixtures giving rise to explosive metal azides. Flush with large volumes of water when disposing of the reagent.

Hazards:
R1 and R2: Hazard Classifications: Not a hazardous substance or mixture.
Pictogram: Not required.
Signal Word: Not required.
Hazard Statements: Not a hazardous substance or mixture.
Precautionary Statements: Not a hazardous substance or mixture.
Refer to the Safety Data Sheet for this product (SDS-L7572) available by calling 1-734-487-8300.

Specimen Collection and Storage
1. Non-hemolyzed serum is recommended. Red cells contain large concentrations of LD.[5]
2. The serum should be removed from the clot promptly.
3. Samples should be assayed soon after collection. LD in serum is reported stable for two to three days at room temperature.[9]
4. Do not freeze or expose the serum to high temperatures (37°C) as this may inactivate thermolabile LD isoenzymes.[10]
5. Specimen collection should be carried out in accordance with NCCLS M29-T2.[11] No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all samples should be considered potentially infectious.

Interferences
1. Certain drugs and substances affect LD activity. See Young, et al.[12]
2. Bilirubin to the level of 20 mg/dl has been found to exhibit negligible interference (≤ 5%) in this assay.
3. Hemolysis has been shown to significantly interfere with the assay at levels as low as 100 mg/dl.

Materials Provided
Lactate Dehydrogenase Buffer (R1) Reagent
Lactate Dehydrogenase Co-Enzyme (R2) Reagent

Pointe
Lactate Dehydrogenase
Reagent Set

Materials Required but not Provided

1. Yumizen C560 Analyzer
2. Yumizen C560 Operation manual
3. Chemistry control, catalog number C7592-100

Limitations

1. Hemolyzed serum will cause falsely elevated serum LD levels.
2. Samples that exceed the linearity limit (1000 U/L) should be diluted with an equal volume of saline and re-assayed. Multiply the results by two to compensate for the dilution.

Calibration

The procedure is standardized by means of the millimolar absorptivity of NADH taken as 6.22 at 340nm under the test conditions described.

Quality Control

The validity of the reaction should be monitored by use of control samples with known normal and abnormal LD values. These controls should be run at least with every working shift in which LD assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values[5]

Male	50-166 U/L (30°C)	80-285 U/L (37°C)
Female	60-132 U/L (30°C)	103-227 U/L (37°C)

Due to a wide range of conditions (dietary, geographical, age, etc.) known to affect reference ranges, it is recommended that each laboratory establish its own reference range.

Performance

1. Assay Range: 2-1000 U/L. Samples that exceed 1000 U/L should be diluted with an equal volume of saline, re-assayed and results multiplied by two.
2. Correlation: A study was performed between the Yumizen C560 and a similar analyzer using this method, resulting in the following:

Method	LDH
N	80
Mean LDH (U/L)	223.4
Range (U/L)	88-866
Standard Deviation	153.8
Regression Analysis	y = 0.964x – 8.1
Correlation Coefficient	0.9995

3. Precision: Precision studies were performed following a modification of the guidelines contained in the NCCLS document EP5-T2.[12]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	111.1	349.1	628.3
Standard Deviation	1.4	2.8	3.4
Coefficient of Variation (%)	1.3%	0.8%	0.5%

Day to Day

Sample	LOW	MID	HIGH
N	40	40	40
Mean	123.6	381.4	696.5
Standard Deviation	1.6	5.2	9.6
Coefficient of Variation (%)	1.3%	1.4%	1.4%

4. Sensitivity: 2 SD Limit of Detection (95% Con Int): 2 U/L

References

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2. Wroblewski, F., LaDue, J.S., Proc. Soc. Exp. Biol. Med. 90:210 (1955).
3. Kubowitz,F., Ott, P., Biochem. 314:94 (1943).
4. Wacker, W.E.C., et al, N. Engl. J. Med. 255:449 (1956).
5. Henry, R.J. et al, Clinical Chemistry; Principles and Technics, 2nd Ed., Hagerstown (MD) Harper & Row, pp. 819-831. (1974).
6. Amador, E., et al, Clin. Chem. 9:391 (1963).
7. Buhl, S.N., et al, Clin. Chem. 23:1289 (1977).
8. Gay, R.J., McComb, R.B., Bowers, G.N., Clinical Chemistry, 2nd Ed., W.B. Saunders Co., 657 (1976).
9. Tietz, N.W., Fundamentals of Clinical Chemistry, 2nd Ed., W.B. Saunders Co., 657,(1976).
10. Kreutzer, H.H., et al, Clin. Chim. Acta 9:64 (1964).
11. NCCLS Document M29-T2, 2nd Ed. (1991).
12. Young, D.S., et al, Clin. Chem., 21:1D (1975).
13. NCCLS Document “Evaluation of Precision Performance of Clinical Chemistry Devices”, 2nd Ed. (1992).

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CHEMISTRY PARAMETERS

Chem:	LDH	No.:	223	Sample Type:	Serum
Chemistry:	Lactate Dehydrogenase			Print Name:	LDH
Reaction Type:	Kinetic			Reaction Direction:	Positive
Pri Wave:	340			Sec Wave:	412
Unit:	U/L			Decimal	0
Blank Time:	0 0			Reaction Time:	56 71

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	7.3 ul	--- ul	--- ul	R1: 120 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2: 30 ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3: --- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	2	1000	Linearity Limit:	0.3
Linearity Range (Decreased)	____	____	Substrate Depletion:	25000
Linearity Range (Increased)	____	____	Mixed Blank Abs:	
R1 Blank Abs:	____	____	Uncapping Time	
Blank Response:	____	____	Reagent Alarm Limit:	
Twin Chemistry:			☐ Enzyme Linear Extension	

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

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Reagent Set

CALIBRATION PARAMETERS

Calibrator Definition

Calibrator:	*	Lot No.:	*
Exp Date:	*		

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	LDH	0	U/L

Calibration Setup

Chem:	LDH		
Calibration Settings			
Math Model:	K Factor		
Factor:	3505	Replicates:	1
Acceptance Limits			
Cal Time:	24	Hour	
Slope Diff:	---	SD:	---
Sensitivity :	---	Repeatability:	---
Deter Coeff:	---		
Auto Calib.			

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF 14-L7572-200

Manufactured by
HORIBA Instruments Incorporated-Pointe Brand
5449 Research Drive Canton, MI 48188

2°C 8°C IVD

Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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CE

Symbol Key

Use by (YYYY-MM-DD) LOT Lot and batch code REF Catalog number
Manufacturer Temperature limitation Consult instructions for use
IVD *In vitro* diagnostic medical device **Rx Only:** Prescription Use Only

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