

LDH (Liquid) Reagent

Catalog #: 43718

for use with the

SDI CA480 Clinical Chemistry System

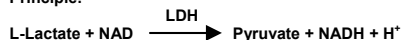
INTENDED USE

For the *in vitro* quantitative determination of lactate dehydrogenase activity in human serum.

METHODOLOGY

The first UV kinetic method for the determination of LDH activity in serum was published by Wroblewski and LaDue¹ in 1955. Their method utilizes the pyruvate to lactate reaction. Wacker et al² in 1956 described a procedure that followed a lactate to pyruvate reaction. The lactate to pyruvate reaction became the preferred reaction³, because of a wider linear range⁴ and no pre-incubation requirement⁵. The SDI Liquid method follows the lactate to pyruvate reaction and has been optimized for greater sensitivity and linearity.

Principle:



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

REAGENT COMPOSITION

Active Ingredients	Concentrations
Reagent 1	
L-Lactate	100 mM
AMP Buffer, pH 8.8	600 mM
Reagent 2	
NAD	6 mM

Concentrations are those in the working reagent.

Precautions

- For *in vitro* diagnostic use only.
- DO NOT pipette by mouth. Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water. For further information, consult the SDI LDH-L Liquid Reagent Material Safety Data Sheet.
- Reagent contains Sodium Azide as a preservative. In a dry state may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.
- Do not use the reagent after the expiration date printed on the kit.

REAGENT PREPARATION

Reagents are supplied in a two vial, ready to use, liquid form.

REAGENT STORAGE

- Store the reagents refrigerated at 2–8°C.
- The reagents are stable until the expiration date, when stored at 2–8°C.
- Working reagent is stable for 2 weeks, when stored at 2–8°C.
- Do not freeze the reagents.

REAGENT DETERIORATION

The reagent should not be used if:

- The initial absorbance of the reagent is greater than 0.8 when read against water at 340 nm.
- The reagent fails to meet stated parameters of performance.

SPECIMEN COLLECTION AND STORAGE

- Non-hemolyzed serum is recommended. Red cells contain large concentrations of LDH.³
- The serum should be removed from the clot promptly.
- Samples should be assayed soon after collection. LDH in serum is reported stable for two to three days at room temperature.⁶
- Do not freeze or expose the serum to high temperatures (37°C) as this may inactivate thermolabile LDH Isoenzymes.⁷

INTERFERENCES

Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out, the following results were obtained:

Hemoglobin:

Do not use hemolyzed samples.

Bilirubin:

No significant interference from bilirubin up to at least 25.8 mg/dL.

Lipemia:

No significant interference from lipemia up to 1086 mg/dL measured as triglycerides.

Certain drugs and substances affect LDH activity. See Young, et al⁸.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- SDI CA480 Clinical Chemistry System
- Deionized water and related equipment, e.g.: pipettes
- Analyzer specific consumables, e.g.: sample cups
- Control material such as those provided by SDI Biomed.

ASSAY PROCEDURE

	System Parameters
LDH-L (Liquid)	
TEMPERATURE:	37°C
WAVELENGTH:	340 nm
ASSAY TYPE:	Rate/Kinetic
DIRECTION:	Increase
SAMPLE / RGT RATIO:	1 : 40
e.g. Sample Vol.	0.025 mL (25mL)
Reagent Vol.	1.0 mL
DELAY/LAG TIME:	1 Min
READ TIME:	1 Min

Procedure Notes:

- The reagent and sample volumes may be altered proportionally to accommodate various instrument requirements.
- Samples with values above 800 U/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

Calculation

One Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute.

$$\text{U/L} = \frac{(A_2 - A_1) / \text{min} \times 1.025 \times 1000}{1 \times 6.22 \times 0.025 \text{mL}} = (A_2 - A_1) \times 6592$$

Where:

$A_2 - A_1$	= Change In absorbance
1.025	= Total reaction volume in mL
1000	= Conversion of U/mL to U/L.
1	= Light path in cm
6.22	= Millimolar absorptivity of NADH
0.025	= Sample volume in mL

Examples:

If absorbance change per minute = 0.03
Then $0.03 \times 6592 = 198 \text{ U/L}$

NOTE:

- If test parameters are altered, the factor has to be recalculated using the above formula.
- To convert to SI Units (nkat/L) multiply U/L by 16.76.

CALIBRATION

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

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with LDH-L values.

EXPECTED VALUES³

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

It is strongly recommended that each laboratory determine the normal range for its particular population.

PERFORMANCE

Linearity:

When run as recommended the assay is linear to 1000 U/L.

Samples with values above 1000 U/L must be diluted 1:1 with normal saline, re-assayed and result multiplied by two.

Method Comparison:

Studies performed between this procedure and one with a similar methodology yielded the following results:

Number of samples pairs:	41
Range of samples:	90 to 929 (U/L)
Correlation Coefficient:	0.9991
Slope:	0.9948
Intercept:	10.9 (U/L)

Precision:

Within Run	Level 1	Level 2
n=40		
Mean (U/L)	157	411
S.D. (U/L)	4.0	5.9
C.V. (%)	2.5	1.4

Total

n=40	Level 1	Level 2
Mean (U/L)	157	411
S.D. (U/L)	4.4	7.1
C.V. (%)	2.8	1.7

References

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- Tietz, N.W., Fundamentals of Clinical Chemistry, 2nd Ed., Philadelphia, W.B. Saunders Co., p. 657, 1976.
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Manufactured for:



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