POINTE SCIENTIFIC, INC.
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LDH - L
Reagent Set

Intended Use
For the *in vitro* quantitative kinetic determination of lactate dehydrogenase activity in serum.

Method History
Wroblewski and LaDue published the first UV kinetic method for the determination of LDH activity in serum in 1955.[1] Their method was based on the classic Kubowitz and Ott assay (1943) utilizing the pyruvate to lactate reaction.[2] In 1956, Wacker et al described a procedure that followed a lactate to pyruvate reaction.[3] The lactate to pyruvate reaction became the preferred reaction, even though the slower of the two, because of a wider linear range and no pre-incubation requirement.[4,5,6] The present method follows the forward reaction and has been optimized for greater sensitivity and linearity.

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

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. The rate is directly proportional to LDH activity in serum.

Reagents
(Concentrations refer to reconstituted reagent.) NAD 7.0 mM, L-Lactate 50 mM, Tris Buffer, pH 9.0 ± 0.1 Non-reactive stabilizers and fillers.

Precautions
This reagent is for *in vitro* diagnostic use only.

Reagent Preparation
Reconstitute reagent with the volume of distilled water stated on the vial. Invert gently to dissolve.

Reagent Storage
1. The dry reagent should be stored refrigerated at (2-8°C).
2. Reconstituted reagent is stable for 21 days when immediately refrigerated at 2-8°C and for eight hours at room temperature.

Reagent Deterioration
The reagent should not be used if:
1. The initial absorbance of the reagent is greater than 0.8 when read against water at 340 nm.
2. The reagent fails to meet stated parameters of performance.

Specimen Collection and Storage
1. Non-hemolyzed serum is recommended. Red cells contain large concentrations of LDH.[4]
2. The serum should be removed from the clot promptly.
3. Samples should be assayed soon after collection. LDH in serum is reported stable for two to three days at room temperature.[7]
4. Do not freeze or expose the serum to high temperatures (37°C) as this may inactivate thermolabile LDH isoenzymes.[8]

Interferences
Certain drugs and substances affect LDH activity. See Young, et al.[9]

Materials Provided
LDH–L reagent.

Materials Required but not Provided
1. Accurate pipetting devices
2. Test tubes/rack
3. Timer
4. Heating bath or block (37°C)
5. Spectrophotometer able to read at 340 nm

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)
1. Reconstitute reagent according to instructions.
2. Pipette 1.0ml of reagent into appropriate tubes and pre-warm at 37°C for five minutes.
3. Zero spectrophotometer with water at 340nm.
4. Transfer 0.025 ml (25ul) of sample to reagent, mix and incubate at 37°C for one minute.
5. After one minute, read and record absorbance (A_1 Reading). Return tube to 37°C for one minute.
6. After exactly one minute, read and record absorbance (A_2 Reading).
7. The change in absorbance (A_2-A_1) multiplied by the factor 6592 (See Calculations) will yield results in IU/L.
8. Samples with values above 800 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

Note: If the spectrophotometer being used is equipped with a temperature controlled cuvette, the reaction mixture may be left in the cuvette while the absorbance readings are taken.

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
1. Use control serums with known normal and abnormal values to monitor the integrity of the reaction.
2. Read the control sera package insert to determine LDH stability and storage instruction.

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

$$IU/L = \frac{(A_2 - A_1) \times 1.025 \times 1000}{1 \times 6.22 \times .025 \text{ ml}} = (A_2 - A_1) \times 6592$$

Where: (A_2-A_1) = Change in absorbance
1.025 = Total reaction volume in ml
1000 = Conversion of IU/ml to IU/L
1 = Light path in cm
6.22 = Millimolar absorptivity of NADH
0.025 = Sample volume in ml

Example: If initial reading (A_1) = 0.450
Final reading (A_2) = 0.480
(A_2-A_1) = 0.03
Then 0.03 x 6592 = 198 IU/L

Note: For SI units (nkat/L), multiply result by 16.76.

Temperature Correction

1. If the reaction is performed at 37°C but reported at 30°C, multiply results by 0.6.
2. If the reaction is performed at 30°C but reported at 37°C, multiply results by 1.7.

Note: Temperature factors give only an approximate conversion and therefore it is suggested that values be reported at the temperature of measurement.

Expected Values[4]

Male 50-166 IU/L (30°C) 80-285 IU/L (37°C)

Female 60-132 IU/L (30°C) 103-227 IU/L (37°C)

Performance

1. Correlation: a study performed between this method and one with a similar methodology yielded a correlation coefficient of 0.996 with a regression equation of y=1.01x – 2.
2. Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
129	3.0	2.3	132	3.0	2.2
340	7.0	2.1	358	9.0	2.5

References

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