

LDH-L (Colorimetric)

LDH-L REAGENT SET (Colorimetric, Endpoint Procedure)

For the quantitative determination of lactate dehydrogenase activity in serum.

INTRODUCTION

The enzyme lactate dehydrogenase (LDH-L) is distributed in tissues particularly heart, liver, muscle, and kidney. The enzyme found in circulation is a mixture of five isoenzymes based on their mobility. Elevated serum levels of LDH-L are found in serum in myocardial infraction, liver disease, renal disease, certain forms of anemia, malignant diseases and progressive muscle dystrophy.^{1,2}

Several colorimetric LDH-L assay method have been developed. Most of these assays are based on the coupling of the reduction of NAD and tetrazolium salts (INT). Nachlas, et al³, described an LDH-L assay using phenazine methosulfate (PMS) as the intermediate electron carrier between NADH and INT. Allain, et al⁴, replaced PMS with the enzyme diaphorase.

PRINCIPLE

LDH catalyzes the oxidation of lactate to pyruvate in the presence of NAD which is subsequently reduced to NADH. The formation of NADH is coupled with the reduction of INT to INT_H catalyzed by the enzyme of diaphorase. INT_H is bright red formazan which is measured photometrically at 500 ± 5 nm. The color intensity is proportional to the LDH-L activity of the sample.

REAGENT COMPOSITION

LDH Substrate: DL-Lactate 75 mM; NAD 5.5 mM, Diaphorase 5 u/ml. Buffer 80 mM; pH = 9.0 ± 0.1 (30°C); Non-reactive stabilizers and fillers.

LDH Color Reagent: An aqueous solution containing INT with preservative.

LDH Calibrator: A lyophilized serum with LDH value provided in each lot. Reconstitute with 5 ml distilled water and swirl to mix. Aliquot into small portions and keep frozen.

PRECAUTIONS

1. For "in vitro" diagnostic use only.
2. Exercise the normal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent.

REAGENT PREPARATION

Reconstitute LDH substrate reagent with volume of distilled water stated on the vial label. Invert gently to dissolve.

REAGENT STORAGE

1. Store reagent at 2 - 8°C.
2. Reconstituted reagent is stable for seven (7) days when refrigerated at 2 - 8°C and for eight (8) hours at room temperature.

REAGENT DETERIORATION

1. If the reagent blank before serum addition exceeds 0.45 at 340 nm, the reagent may have deteriorated.
2. Failure to obtain accurate results in the assay of control materials may indicate reagent deterioration.

SPECIMEN COLLECTION AND HANDLING

1. Serum with any visible hemolysis can not be used because of the contamination of this sample with large amount of LDH-L released from the erythrocytes.³
2. Serum should be separated from the clot promptly.
3. Samples should be assayed soon after collection. LDH-L in serum is reported stable for two to three days at room temperature.²
4. The liver LDH-L is particularly labile and is destroyed if frozen and thawed.⁵

INTERFERENCES

1. Oxalate, oxamates, and EDTA will inhibit LDH-L.
2. Young, et al., gave a list of drugs and other substances interfere with the determination of LDH-L activity.⁶

MATERIALS PROVIDED

LDH-L substrate, LDH-L Color Reagent and LDH-L Calibrator.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Accurate pipeting devices.
2. Test tubes and rack.
3. Timer.
4. Heating bath or block (37°C).

5. 0.1 N Hydrochloric Acid.

PROCEDURE (MANUAL, ENDPOINT)

1. Pipette 0.5 ml of LDH-substrate into vials labeled reagent blank, calibrator, control, and unknown(s).
2. Add 0.1 ml (100 µl) of LDH Color reagent to all vials, mix and prewarm at 37°C for three (3) minutes.
3. At time intervals, add 0.020 ml (20 µl) of sample to its respective vial, mix and incubate at 37°C for exactly ten (10) minutes.
4. Using the same timed interval sequence, add 2.0 ml of 0.1 N Hydrochloric acid to the reaction vials to stop the reaction. Mix all tubes.
5. Set the wavelength of the photometer at 500 nm and zero instrument with reagent blank. Read and record absorbance of all vials. (Wavelength range: 500-520).

* USE TC - MUTI PURPOSE CALIBRATOR TO REPLACE STANDARD.

CALCULATION

Use the absorbance readings of the calibrator and unknown(s) to calculate LDH-L values as follows:

$$\frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{concentration of Calibrator (IU/L)} = \text{LDH in unknown (IU/L)}.$$

Example of Calculation:

Assume that the calibrator has a concentration of 150 IU/L and that it gave an absorbance of 0.240, while the unknown gave an absorbance of 0.350. The LDH concentration of the unknown may then be calculated as follows:

$$\frac{0.350}{0.240} \times 150 \text{ IU/L} = 218 \text{ IU/L}.$$

PROCEDURE LIMITATION

1. The procedure measures total lactic dehydrogenase irrespective of its tissue or organ of origin.
2. The reaction temperature must be maintained to within ± 0.1 °C, during the assay.

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with LDH-L values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction or procedural errors.

TEMPERATURE CORRECTION⁷

1. If the assay is performed at 37°C but is to be reported at 30°C; multiply the results by 0.6

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

EXPECTED VALUES⁷

Males 80 - 285 IU/L (37°C)
Females 103 - 227 IU/L (37°C)

It is strongly recommended. that each laboratory establish its own normal range.

PERFORMANCE

1. Linearity: 800 IU/L
2. Comparison: Studies between the present method and with an optimized LDH, UV method yield a correlation coefficient of 0.99 and a regression equation of $y = 1.00x \text{ (UV)} + 3.71$.
3. Precision studies.

<u>Mean (mg/dl)</u>	Within Run	
	<u>S.D.</u>	<u>C.V.%</u>
145	9	6
387	18	5

<u>Mean(mg/dl)</u>	Run to Run	
	<u>S.D.</u>	<u>C.V.%</u>
146	12	8
380	22	6

REFERENCES

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