

# LDH

(L→P, Kinetic Method)

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**Ref.** CC1-LDK.014, 20x1.1 ml  
CC2-LDK.14U, 20x3 ml

## INTENDED USE

Reagent kit for quantitative estimation of Lactate Dehydrogenase activity in serum or plasma.

## PRODUCT HIGHLIGHTS

- One of the most reliable LDH reagent in the market. Most of the Reagent kits have moisture (converting β-NADH to α-NADH) problems.
- Suitable pack sizes and corresponding suitable reconstituted stability.
- Most economical with no wastage of reagents.
- Necessary when CK-NAC/CK-MB is inconclusive due to macro CK-BB etc.
- More reliable reagent for CHD follow up after 4 to 6 days.

## INTRODUCTION

Lactate dehydrogenase is a cytoplasmic enzyme distributed very widely in the body. It is found in organs like heart, liver, kidney and skeletal muscle. Elevated LDH levels in serum are observed in several hemolytic, neoplastic, cardiac, skeletal muscle and renal diseases. These may also be found in destructive renal disease, progressive muscular dystrophy, megaloblastic anaemia, liver cirrhosis, hepatitis, hepatic metastasis, hepatoma and pulmonary embolism.

Lactate to pyruvate in the presence of lactate dehydrogenase is a reversible reaction. However the L→P reaction is favoured at a pH of 7.4 and this reaction utilises NAD as a coenzyme which is more stable compared to NADH.

LDH L→P is formulated based on the procedure of Gay, Bowers and Mc Combs (1968)

## DIAGNOSTIC SIGNIFICANCE

LDH is one of the five (i.e. CK/CPK, CK-MB, GOT/AST, LDH and HBDH) cardiac panel enzymes. Estimation of these enzymes is a powerful tool at the onset of CHD and equally useful in monitoring progress status of the patients.

## PRINCIPLE

LDH catalyzes the oxidation of lactate to pyruvate accompanied by the simultaneous reduction of NAD to NADH. LDH activity in serum is proportional to the increase in absorbance due to the reduction of NAD.



## PRESENTATION

Store all reagents at 2-8°C	No. of Bottles	
	20 x 1.1 ml	20 x 3 ml
• 1 LDH (Coenzyme)	20	20
• 2 LDH (Buffer)	1	1

## FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• NAD	5 mmol/L
• Lactate	50 mmol/L
• Buffer	100 mmol/L

pH 9.3 ± 0.5 at 25°C

Also contains non-reactive fillers & stabilizers.

## PRECAUTION

LDH (L→P) is for *IN-VITRO* diagnostic use only.  
Reagent Contains Sodium Azide, DO NOT INGEST.

## PREPARATION OF WORKING REAGENT

### For 20 x 1.1 ml

Reconstitute one vial of 1 LDH with 1.1 ml of 2 LDH (Buffer). Mix gently to dissolve the contents. Use after 5 minutes.

### For 20 x 3 ml

Reconstitute one vial of 1 LDH with 3 ml of 2 LDH (Buffer). Mix gently to dissolve the contents. Use after 5 minutes.

## REAGENT STORAGE AND STABILITY

LDH (L→P) reagents are stable at 2- 8°C until the expiry date stated on the label. Reconstituted reagent is stable at 2- 8°C for 30 days.

## SPECIMEN COLLECTION

Fresh, clear, serum with no hemolysis is essential.

## REACTION PARAMETERS

- Type of Reaction : Kinetic/Increasing OD
- Wavelength : 340 nm
- Flowcell Temperature : 37°C
- Delay Time : 60 Seconds
- Interval Time : 30 Seconds
- Number of Readings : 4
- Reagent Volume : 1.0 ml.
- Sample Volume : 50 µl (0.05 ml)
- Factor : 3376
- Zero setting with : Distilled Water
- Path length : 1.0 cm

## TEST PROCEDURE

Pipette Into Test Tubes	TEST
Working Reagent (ml)	1.0
Sample (ml)	0.05

Mix and read first absorbance of the test exactly at one minute & thereafter at 30, 60 & 90 seconds at 340 nm. Determine the mean change in absorbance per minute and calculate test results.

## TEST RESULTS

LDH activity =  $\Delta A/\text{min} \times F$

$$\text{Where } F = \frac{1}{6.22} \times \frac{T.V.}{S.V.} \times 1000 = 3376$$

T.V. = Total Volume = 1.05 ml.

S.V. = Sample Volume = 0.05 ml.

6.22 = Millimolar Extinction Coefficient of NADH at 340 nm.

## NORMAL VALUES

Adults: 114 - 240 IU/L at 37°C

## LINEARITY

The method is linear upto 2000 IU/L . For samples with values higher than 2000 IU/L, dilute the samples suitably with 0.9% saline and repeat the assay. Apply proper dilution factor and calculate the final results.

## NOTE

For laboratories using instruments with cuvette capacity more than/ less than 1.0 ml, sample and working reagent volumes should be proportionately increased/decreased.

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## REFERENCES

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3. Lum, G. Gambino, S.R. Am. J. Clin Pathod. 61 (108) 1974.



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