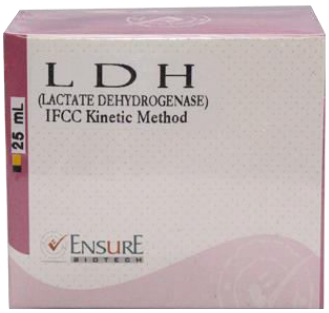
LDH-P
IFCC METHOD



INTENDED USE :

This reagent kit is intended for in vitro quantitative determination of Lactate Dehydrogenase (LDH) activity in serum.

CLINICAL SIGNIFICANCE :

Lactate dehydrogenase (LDH) is present in every cell, it is a tetramere molecule which is a combination of two different tissue com nents (M-muscle, H-heart). There are five different isoenzymes: LD -1: LD -2 LDH-3: LDH-4: LDH-5 = 20: 34: 23: 12 11. The serum activity is mainly composed of LDH-l , LDH-2 derived from the myocardium and red blood cells, and LDH-5 derived from the ver. The activities of isoenzymes are different in cases of certain substrates. The inhibitors and pH sensitivities are different The various fractions were determined using chromatogra hy in the past but more recently electrophoresis is the method 0 choice. The ratio of isoenzymes indicates certain disease states. The enzyme activity significantl increases 8-12 hours following a myocardial infarction and declines a er 4-5 days. There is an increase in liver diseases, in certain anaemia and tissue injuries. The enzyme catalyses the Pymvate l Lactate transformation at optimal pH.

PRINCIPLE:

LDH catalyses the transformation of Pyruvate to Lactate in Tris buffer with NaCl in the presence of NADH coenzyme. The transformation of NADH to NAD° is accompanied by a decrease in absorbance at 340nm. The change in absorbance correlates with the LDH activity in the serum.

$$\text{Pyruvate} + \text{NADH} + H^+ \rightarrow \text{Lactate} + NAD^+$$

Reagent Composition:

Reagent1	:	Enzyme Reagent
Reagent2	:	Substrate Reagent

MATERIALS REQUIRED BUT NOT PROVIDED

Clean & Dry Glassware.

Micropipettes & Tips.

Colorimeter or Bio-Chemistry Analyzer

SAMPLES:

Serum free of hemolysis

WORKING REAGENT PREPARATION 81 STABILITY:

Mix 4 Volume of Reagent 1 , with 1 Volume of Reagent 2.

Working Reagent is stable for 30 days at 28°C.

GENERAL SYSTEM PARAMETERS :

Reaction type	Kinetic Reaction (Decreasing)
Wave length	40nm
Light Path	1 Cm
Reaction Temperature	37°C
Blank/Zero Setting	With Distilled Water
Reagent Volume	1 ml
Sample Volume	20 µl
Lag/Delay Time	60 Sec.
Read Time	120 Sec.
interval Time	60 Sec.
Factor	8100
Low Normal at 37°C	230 U/I
High Normal at 87°C	460 U/I
Linearity	1800 U/l
Reagent Absorbance Limit	>08
Max.AAbs/Min	0.222

ASSAYPROCEDURE:

Working Reagent	1000 µl
Sample	20 µl

Mix and after 60 second incubation, measure the decrease in . absorbance every minute during 3 minutes at 37°C. Determine the ΔA/min.

CALCULATION :

At 340 nm with 1 cm Light path

LDH Activity (U/I) = ΔA/min. x8100

LINEARITY:

Reagent is Linear up to 1890 U/I
Dilute the sample appropriately and re-assay it
LDH Activity exceeds 1800 U/I or ΔAbs / mm
Exceeds 0.222. Multiply result with dilution factor.

REFERENCE NORMAL VALUE:

230 to 460 WI
The reference values are only indicative in nature. Every laboratory should establish IIS own normal ranges.

QUALITY CONTROL:

For accuracy it is necessary to run known controls with every assay.

LIMITATION 8t PRECAUTIONS:

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the lot.
3. Be ore the assay bring all the reagents to room temperature.
4. Avoid contamination of the reagent during assay process.
5. Use clean glassware free from dust or debris.
6. Reagent to sample ratio as mentioned here above must be strictly observed as any change in to it will effect the factor.

BIBLIOGRAPHY:

BergmeyerHU. J. Clin. Chem. Clin. Biochem. 13.2269(1975). Howell B.F. 3 al. Clrn. Chem. 25,269 (1979)