LDH - P
IFCC METHOD

ORDER INFORMATION

CODE : R1- 4X20 ML + R2- 2X10 ML (100ml)

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 components (M-muscle, H-heart). There are five different isoenzymes: LDH-1: LDH-2: LDH-3: LDH-4: LDH-5 = 20: 34: 23: 12: 11. The serum activity is mainly composed of LDH-1, LDH-2 derived from the myocardium and red blood cells, and LDH-5 derived from the liver. The activities of isoenzymes are different in cases of certain substrates. The inhibitors and pH sensitivities are different. The various fractions were determined using chromatography in the past but more recently electrophoresis is the method of choice. The ratio of isoenzymes indicates certain disease states. The enzyme activity significantly increases 8-12 hours following a myocardial infarction and declines after 4-5 days. There is an increase in liver diseases, in certain anaemia and tissue injuries. The enzyme catalyses the Pyruvate / 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 340 nm. The change in absorbance correlates with the LDH activity in the serum.

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

REAGENT COMPOSITION :

Reagent 1 : Enzyme Reagent
Reagent 2 : 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 & STABILITY :

Mix 4 Volume of Reagent 1, with 1 Volume of Reagent 2.
Working Reagent is stable for 30 days at 2 - 8°C.

GENERAL SYSTEM PARAMETERS :

Reaction type	Kinetic Reaction (Decreasing)
Wave length	340 nm
Light Path	1 Cm
Reaction Temperature	37°C
Blank / Zero Setting	With Distilled Water
Reagent Volume	1ml
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/l
High Normal at 37°C	460 U/l
Linearity	1800 U/l
Reagent Absorbance Limit	>0.8
Max. Δ Abs / Min	0.222

ASSAY PROCEDURE :

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 1cm Light path

LDH Activity (U/l) = ΔA/min. x 8100

LINEARITY :

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

REFERENCE NORMAL VALUE :

230 to 460 U/l

The reference values are only indicative in nature. Every laboratory should establish its own normal ranges.

QUALITY CONTROL :

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

LIMITATION & 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 kit.
3. Before 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 :

Bergmeyer HU. J. Clin. Chem. Clin. Biochem. 13, 2269 (1975).
Howell B.F. and al. Clin. Chem. 25, 269 (1979)

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