LDH (P-L) KIT

(DGKC Method)

(For invitro diagnostic use only)

CE

INTENDED USE

LDH (P - L) Kit is used for the determination of Lactate Dehydrogenase Activity in serum.

SUMMARY

LDH is found in many body tissues particularly heart, liver, skeletal muscle, kidney and RBC'S. LDH is found in the form of isoenzymes based on their electophoretic mobility with each isoenzyme being primarily from different organs. Increased levels are found in myocardial infarction, pulmonary diseases, hepatic diseases, hemolytic anemias, renal diseases and muscular dystrophy.

PRINCIPLE

Lactate dehydrogenase catalyzes the reduction of pyruvate with NADH to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance, which is proportional to the LDH Activity in the sample.

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

EXPECTED VALUES

Serum : 230 - 460 U/L at 37°C

It is recommended that each laboratory establish its own normal range representing its patient population.

PRESENTATION

REF	1102160025	1102160275	1102160075
Pack Size	25 ml	2 x 75 ml	75 ml
L1 Buffer Reagent	20 ml	2 x 60 ml	60 ml
L2 Starter Reagent	5 ml	2 x 15 ml	15 ml

COMPOSITION

Tris Buffer 100mM; Pyruvate 1.2mM; NaCl 200mM; NADH 0.18mM; Non Reactive Stabilizers and Preservatives.

STORAGE / STABILITY

Contents are stable at 2-8°C till the expiry mentioned on the labels.

REAGENT PREPARATION

Reagents are ready to use.

Working reagent: For sample start assays a single reagent is required. Pour the contents of 1 bottle of L2 (Starter Reagent) into 1 bottle of L1 (Buffer Reagent). This working reagent is stable for at least 1 week when stored at 2-8°C.
Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of L1 (Buffer Reagent) and 1 part of L2 (Starter Reagent). Alternatively 0.8 ml of L1 and 0.2 ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

SAMPLE MATERIAL

Serum. Free from hemolysis. Total LDH is reported to be stable in serum for 1-3 days at 2-8° C. **Freezing inactivates the liver isoenzyme.**

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.
This product requires the handling of human specimens. It is recommended that all human sourced material are considered potentially hazardous and are handled in accordance with the OSHA standard on blood borne pathogens.
Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

MATERIALS REQUIRED BUT NOT PROVIDED

Photometer analyzer with standard thermostatic cuvette holder, micropipette and appropriate laboratory equipment.

PROCEDURE

Wavelength / filter : 340 nm
Temperature : 37° C / 30° C / 25° C
Light path : 1 cm

Substrate Start Assay:
Pipette into a clean dry test tube labelled as Test (T):

Addition Sequence	(T) 25° C / 30° C	(T) 37° C
Buffer Reagent (L1)	0.8 ml	0.8 ml
Sample	0.05 ml	0.02 ml
Incubate at the assay temperature for 1 min. and add		
Starter Reagent (L2)	0.2 ml	0.2 ml

Mix well and read the initial absorbance A_0 after 1 min. and repeat the absorbance reading after every 1, 2, & 3 mins. Calculate the mean absorbance change per min. (∆A / min).

Sample Start Assay:
Pipette into a clean dry test tube labelled as Test (T):

Addition Sequence	(T) 25° C / 30° C	(T) 37° C
Working Reagent	1.0 ml	1.0 ml
Incubate at the assay temperature for 1 min. and add		
Sample	0.05 ml	0.02 ml

Mix well and read the initial absorbance A_0 after 1 min. and repeat the absorbance reading after every 1, 2, & 3 mins. Calculate the mean absorbance change per min. (∆A / min).

CALCULATIONS

Substrate / Sample start
LDH Activity in U/L 25° C / 30° C = ∆A / min. x 3333
37° C = ∆A / min. x 8095

QUALITY CONTROL

The following process is recommended for QC during the assay of LDH (P-L). *Define and establish acceptable range for your laboratory.

1. Two levels of control (Normal and Abnormal) are to be run on a daily basis.
2. If QC results fall outside acceptance criteria, recalibration may be necessary.
3. Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity:
The procedure is linear upto 2000 U/L at 37° C. If the absorbance change (∆A / min.) exceeds 0.250, use only the value of the first 2 mins. to calculate the result or dilute the sample 1+9 with normal saline (NaCl 0.9%) and repeat the assay (Results x 10).

Limit of detection:
The limit of detection for LDH (P-L) is 8 U/L.

Interferences:
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Precision:
Precision studies were performed with two controls using NCCLS protocol EP5-A. The results of the precision studies are shown below:

Sample	Within-run		Between-run		Total	
	Mean	CV%	Mean	CV%	Mean	CV%
Control 1	331.86	1.80	332.54	1.71	664.4	3.51
Control 2	524.01	1.22	527.69	0.86	1051.7	2.08

Method comparison:
Comparative studies were done to compare our reagent with another commercial LDH (P-L) Assay. No significant differences were observed. Details of the comparative studies are available on request.

TEMPERATURE CONVERSION FACTORS

Assay Temperature	Desired Reporting Temperature		
	25° C	30° C	37° C
25° C	1.00	1.33	1.92
30° C	0.75	1.00	1.44
37° C	0.52	0.70	1.00

NOTE

In vitro diagnostic reagent for laboratory and professional use only Not for medicinal use. The reagent contain sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water. Only clean and dry glassware must be used. Samples having a high activity show a very low initial absorbance as most of the NADH is consumed prior to the start of measurement. If this is suspected then dilute the sample and repeat the assay.
The working reagent or the combined reagent should have an absorbance above 1.000 against distilled water at 340 nm. Discard the reagent if the absorbance is below 1.000.
RBC's have a very high LDH content and hence hemolysed samples should not be used. The reagent may be used in several automated analyzers. Instructions are available on request. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

1. Recommendations for the measurement of LDH in human serum at 30°C. Ann. Biol. Chem. (1982) 40: 87.
2. Weishaar H. D. et. al. (1975) Med. Welt 26:387 Calculated Values of Section. Please Note "Temperature Conversion Factors".
3. Data on file: Coral Clinical Systems.

System Parameters

Reaction	: U.V. Kinetic	Interval	: 60 Sec.
Wavelength	: 340 nm	Sample Vol.	: 0.02 ml
Zero Setting	: Distilled Water	Reagent Vol.	: 1.00 ml
Incub. Temp.	: 37° C	Standard	: ---
Incub. Time	: ---	Factor	: 8095
Delay Time	: 60 Sec.	React. Slope	: Decreasing
Read Time	: 180 Sec.	Linearity	: 2000 U/L
No. of read.	: 4	Units	: U/L

8°C / 2°C Store at 2-8°C	Manufacturer	IVD *In vitro* Diagnostic Medical Device	L1 Buffer Reagent	Mod. IFCC Mod. IFCC Method
Use by (Last day of stated month)	Consult Instructions for use	LOT Batch Number	L2 Starter Reagent	This way up
Date of Manufacture	REF Catalogue Number	EC REP Authorised Representative in the European Community		

Manufactured by:

Coral Clinical Systems
A Division of Tulip Diagnostics (P) Ltd.
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