INTRODUCTION

1. *Infinite* Liquid LDH is a reagent set for determination of lactate dehydrogenase activity in serum and plasma based on **UV - Kinetic method.**
2. *Infinite* Liquid LDH is a **ready-to-use, two liquid reagent system.**
3. *Infinite* Liquid LDH estimates LDH activity in **2½ minutes** at 37°C.
4. *Infinite* Liquid LDH is **linear** upto 2000 IU/l.
5. *Infinite* Liquid LDH is a high stability reagent.
6. *Infinite* Liquid LDH can be used on any **Spectrophotometer, Discrete semiautomated and Automated analyzers.** Programme can be designed for any specific analyzer upon request.

PRINCIPLE

Lactate dehydrogenase (LD or LDH) catalyzes the reduction of pyruvate by NADH to form lactate and NAD^+. The catalytic concentration is determined from the rate of decrease of NADH measured at 340 nm.

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

PREPARATION OF WORKING SOLUTION

Prepare working solution by mixing **Reagent R_1** and **Reagent R_2** in the ratio **4:1** as per requirement.

REAGENT STORAGE & STABILITY

The reagent kit should be stored at 2- 8°C and is stable till the expiry date indicated on the label.

R_1 and R_2 are stable till expiry at 2-8°C.

The working solution (4 R_1 + 1 R_2) is stable for 30 days at 2-8°C.

DO NOT FREEZE THE REAGENT. Contamination of the reagents should be strictly avoided.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
• Tris buffer, pH 6.8	100 mmol/l
• EDTA	0.07 gm/l
• NADH	0.28 mmol/l
• Sodium pyruvate	1.20 mmol/l
• Sodium chloride	160 mmol/l

SPECIMEN COLLECTION & PRESERVATION

Collect the specimen in a clean & dry container. Although serum is preferred, plasma with Heparin or EDTA can be used. Hemolyzed samples should not be used since LDH activity in erythrocytes is 160 fold higher than in serum. The serum should be separated from the clot promptly. Samples should be assayed soon after collection. LDH is stable in serum or plasma for four days at 2 - 8°C. Do not freeze or expose the serum to high temperature as this may inactivate thermolabile LDH isoenzymes.

PROCEDURE

☐ Reaction type	UV - Kinetic
☐ Reaction direction	Decreasing
☐ Wavelength	340 nm.
☐ Flowcell temperature	37°C
☐ Zero setting with	Distilled water
☐ Delay time	60 seconds
☐ No. of readings	4
☐ Interval	30 seconds
☐ Blank absorbance limit	≥ 1.000 Abs.
☐ Sample volume	0.02 ml (20 µl)
☐ Working solution volume (4 R_1 : 1 R_2)	1.0 ml
☐ Factor	8109
☐ Linearity	2000 IU/l

Manual assay procedure

Prewarm at 37°C the required amount of working solution before use. Perform the assay as given below :

1.0 ml procedure

Serum / Plasma	0.02 ml (20 µl)
Working solution	1.0 ml (800 µl R_1 + 200 µl R_2)

Mix thoroughly and transfer the assay mixture immediately to the thermostated cuvette and start the stop watch simultaneously. Record the first reading at 60th second and subsequently three more readings with 30 seconds interval at 340 nm.

Calculation:

Calculate the average change in absorbance per minute

(Δ Abs./30 seconds x 2)

Activity of LDH in IU/l = Δ Abs./min x 8109

LD-2009-03-001

EXPECTED VALUES

		25°C	30°C	37°C
IU/l	Adults	120 - 240	161 - 322	240 - 480
µkat/l	Adults	2.00 - 4.00	2.68 - 5.37	4.00 - 8.00

The following factors are used for conversion:

From 25°C to 30°C: 1.34

From 25°C to 37°C: 2.00

NOTE :

Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own normal range

PROCEDURE LIMITATIONS

1. Working reagent is considered unsatisfactory & should not be used if its absorbance is less than 1.000 at 340 nm. against distilled water.
2. If LDH activity is above 2000 IU/l then dilute the specimen suitably with normal saline. In such case the results obtained should be multiplied by the dilution factor to obtain correct LDH activity.

QUALITY CONTROL

To ensure proper quality control, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. Quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

Liquid dispensing facility

REFERENCES

1. Thomas L. ***Clinical Laboratory Diagnostics*** 1st ed. Frankfurt: TH - Books Verlagsgesellschaft; 1998:89-94.
2. NCCLS Document ***"Evaluation of Precision Performance of Clinical Chemistry Devices",*** 2nd ed. (1992).
3. Moss, D.W., Henderson, A.R., Clinical Enzymology In: Burtis, C.A., Ashwood, E.R., editors. ***Tietz Textbook of Clinical Chemistry.*** 3rd ed. Philadelphia. W.B. Saunders Company; 1999:617-721.
4. In house test data. ***Accurex Biomedical Pvt. Ltd.,*** 2005.

IVD	*In Vitro* Diagnostic Use		Date of Manufacturing
	Consult Instructions for use		Use by (YYYY-MM-DD)
REF	Catalogue Number		Temperature Limitation
LOT	Batch Code		Manufacturer

CE European Conformity

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