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LDH-L

CE

STABLE LIQUID REAGENT

Cat. No.:	47561	47562	47563
	100 ml	600 ml	10x20 ml
	(1x50 ml + 1x50 ml)	(1x300 ml+ 1x300 ml)	(10x10 ml+ 10x10 ml)

Reagent kit for the determination of lactate dehydrogenase activity in serum.

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 Lactate / Pyruvate transformation at optimal pH.

Principle

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

LDH=Lactate dehydrogenase

Reference values

Serum LDH-L activity: 62-155 U/l (1,03-2,58 µkat/l)

It is recommended that each laboratory should assign its own normal range.

Reagents

1. Reagent 1 (R1)

Buffer, pH=9.0	
2-Methyl-2-Amino-1-Propanol	600 mmol/l
L-Lactate	100 mmol/l

2. Reagent 2 (R2)

NAD	6 mmol/l

Note

The R1 contains sodium azide (0.1 %). To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent.

Sample

Serum free of haemolysis.

PROCEDURE

Preparation and stability of working reagent

Mix 1 volume of R1 with 1 volume of R2.

Stability: 2 - 8 °C 4 weeks
20-25 °C 7 days

If the absorbance of working reagent is higher than 0.7 at 334 nm the reagent can not be used.

Assay conditions

Wavelength:	340 (334-365) nm
Temperature:	37 °C
Cuvette:	1 cm light path
Read against:	reagent blank
Method:	kinetic (increasing)

Pipette into cuvette

	Blank	Sample
Working reagent	1 ml	1 ml
Distilled water	50µl	
Sample		50µl

Mix and after 3 minutes incubation measure the change of absorbance per minute (ΔA/min) during 3 minutes.

Calibration

S1: Distilled water
S2: Roche C.F.A.S. (Calibrator for automated system) or
Randox Calibration Serum Level I

Calibration frequency

Two point calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} x C_{standard} = C_{sample}$$

A = Absorbance
C = Concentration

Calculation using factor

U/l = ΔA Sample/minute x 3333 (334 nm); µkat/l= ΔA Sample/minute x 55,6
U/l = ΔA Sample/minute x 3400 (340 nm); µkat/l= ΔA Sample/minute x 56,7

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

Linearity

The test is linear up to 1000 U/l (16,67 µkat/l)

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 3.3 U/l (0,055 µkat/l) LDH-L activity at 334 nm.

Accuracy

Obtained values of control serum samples with known amount of LDH-L activity fall within +/- 10%.

Precision

	Repeatability
	CV%
Serum	<5

NOTE

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For *in vitro* diagnostic use only.

The following symbols are used on labels

IVD For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

LOT Batch Code

REF Code

Bibliography

Buhl, S.N. and Jackson, K.Y. Clin. Chem., 24/5. (1978), 828.