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LDH-P

CE

STABLE LIQUID REAGENT

Cat. No.:	46461	46462	46463
	100 ml	600 ml	10x20 ml
	(1x50 ml + 1x50 ml)	(1x300 ml+ 1x300 ml)	(10x10 ml+ 10x10 ml)

Kinetic determination of the lactate dehydrogenase activity in serum based upon DGKC recommendations.

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 pH=7.5 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.

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

Reference values

Serum LDH-P activity: 240-480 U/l (4,0-8,0 µkat/l)

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

Reagents

1.Reagent (R1)

Phosphate buffer, pH=7.50	56 mmol/l
Pyruvate	1.6 mmol/l

2.Reagent (R2)

NADH	240 µmol/l

Precautions

Discard cloudy reagent. Avoid contamination by using clean laboratory material (pipettes, plastic vials for analyzers,...).

These reagents contain 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

• One-reagent procedure:

Mix 1 volume of reagent 1 (Rl) with 1 volume of reagent 2 (R2).

Stability :	at 20-25°C:	1 week
	at 2-8 °C:	4 weeks

• Two-reagent procedure:

The reagents are ready for use.

If the absorbance of working reagent is lower than 1.1 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:	distilled water
Method:	kinetic (decreasing)

- **One-reagent procedure:**

Working reagent	1 ml
Sample	25µl

Mix and after 1 minute incubation, measure the change of optical density per minute (ΔA/min) during 3 minutes.

- **Two-reagent procedure:**

R1	1 ml
Sample	50µl

Mix and after 1 minute incubation, add:

R2	1 ml

Mix and after 1 minute incubation, measure the change of absorbance per minute (ΔA/min) during 3 minutes.

Calibration: (37°C, DGKC method)

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 using calibration

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

A = Absorbance,

C = Concentration

Calculation using factor

334 nm: Activity (U/l) = ΔA/min x 8252; (µkat/l) = ΔA/min x 137

340 nm: Activity (U/l) = ΔA/min x 8450; (µkat/l) = ΔA/min x 141

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

The following data were obtained using the Olympus 600 analyzer (37°C).

Linearity

The test is linear up to 1200 U/l (20µkat/l) LDH-P activity.

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 8.252 U/l (0,138µkat/l) LDH-P activity at 334 nm.

Precision

	Reproducibility		
	Average activity (U/l)	SD	CV%
sample I	344.6	3.41	0.99
sample II	523.6	17.13	3.27

Correlation

Comparative studies were done to compare our reagent with another commercial LDH-P reagent.

The results from these studies are detailed below.

Correlation coefficient: r = 0.9987

Linear regression: y (U/l) = 1.042x+13.1

(x= other commercial reagent, y= own reagent).

Specificity

Bilirubin 855µmol/l (50mg/dl), lipid 1000mg/dl, glucose 55.5 mmol/l (1000mg/dl) and ascorbic acid 2.84 mmol/l (50mg/dl) don't interfere with the assay up to the given levels.

Note

Haemolysis interferes with the test.

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

Ann. Biol. Clin. 1982; 40:123.