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ALPHA-HBDH

FREEZE DRIED

Cat. No.: 42921

15x20 ml

42922

60x20 ml

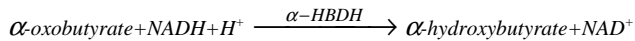


Reagent kit for determination of α -hydroxybutyrate dehydrogenase (α -HBDH) activity in serum. DGKC method.

There is a significant difference between affinities of lactate dehydrogenase isoenzymes for α -hydroxybutyrate as substrate. High affinity of LDH-1 for this substrate permits a rapid, differentiated determination of the enzyme activity. Increased activities are found associated with myocardial infarction.

Principle

LDH-1 isoenzyme in the presence of NADH and H^+ converts α -oxobutyrate substrate into α -hydroxybutyrate while NAD^+ is formed. The rate of decrease in absorbance is proportional to the α -hydroxybutyrate dehydrogenase activity.



Reference values

Serum α -HBDH activity: 80-220 U/l (1,33-3,67 μ kat/l)

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

Reagents

1.Reagent (R1)

Phosphate buffer, pH=7.50 50 mmol/l

α -oxobutyrate 3 mmol/l

2.Reagent (R2)

NADH 0.18 mmol/l

Samples

Serum free of haemolysis.

PROCEDURE

Preparation of working reagent

Dissolve the content of a vial of reagent (R2) in an appropriate amount of reagent (R1).

Stability of working reagent

at 20-25°C: 5 days

at 2-8°C: 4 weeks

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 pathway

Method: kinetic (decreasing)

Pipette into cuvette

Working reagent	3 ml
Sample or control	100 μ l

Mix and after one minute incubation read the change of absorbance in every minute for 3 minutes. Determine the change of absorbance per minute ($\Delta A/\text{min}$).

Calibration: (37°C, DGKC method)

S1: Distilled water

S2: Roche C.F.A.S. (Calibrator for automated system) or

Randox Calibration Serum Level I or

Randox Calibration Serum Level II

Calibration frequency

Two point calibration is recommended

- after reagent lot change,

- as required following quality control procedures.

Calculation using calibration

$$\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}$$

A = Absorbance, C = Concentration

Calculation using factor

340 nm: $\Delta A/\text{min} \times 6573 = \text{U/l}$; $\Delta A/\text{min} \times 109,6 = \mu\text{kat/l}$

334 nm: $\Delta A/\text{min} \times 6700 = \text{U/l}$; $\Delta A/\text{min} \times 111,7 = \mu\text{kat/l}$

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 490 U/l (8,17 μ kat/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 6.700 U/l (0,11 μ kat/l) α -HBDH activity at 334 nm.

Precision

	Reproducibility		
	Average activity	SD	CV%
sample I	155.3	5.61	3.61
sample II	260.0	5.79	2.23

Correlation

Comparative studies were done to compare our reagent with another commercial α -HBDH reagent.

The results from these studies are detailed below.

Correlation coefficient: $r=0.9994$

Linear regression: $y (\text{U/l}) = 1.043x - 2.145$

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

Specificity

Bilirubin 855 $\mu\text{mol/l}$ (50 mg/dl), lipid 1000mg/dl, glucose 41.6 mmol/l (750mg/dl) and ascorbic acid 2.84 mmol/l (50mg/dl) don't interfere with the assay up to the given levels.


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

 For *in vitro* diagnostic use

 Use by (last day of the month)

 Temperature limitation

 Batch Code

 Code

Bibliography

Rec. GSCC (DGKC): *J. Clin. Chem. Clin. Biochem*, 8: 658, (1970)

Rec. GSCC (DGKC): *J. Clin. Chem. Clin. Biochem*, 10:182, (1970).