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

DGCk method.

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

Principle

LDH-I isoenzyme in the presence of NADH and H⁺ substrate permits a rapid, differentiated determination of the enzyme activity.

Stability: at 2-8 °C: 1 week

Mix 1 volume of reagent 1 (R1) with 1 volume of reagent 2 (R2).

One-reagent procedure:

Preparation and stability of working reagent

Calibration:

- Standard: Roche C.F.A.S. (Calibrator for automated system) or
- Randox Calibration Serum Level II
- Serum free of haemolysis.

Calculation using calibration factor

- One reagent procedure

HBDH activity at 334 nm.

NOTE

Reagents and ascorbic acid 2.84 mmol/l (50mg/dl) don't interfere with the assay up to the standard range.

Samples

Serum free of haemolysis.

Haemolysis interferes with the test.

PROCEDURE

Preparation and stability of working reagent

- One-reagent procedure:

Mix 1 volume of reagent 1 (R1) with 1 volume of reagent 2 (R2).

Stability: at 20-25 °C: 1 week

If the absorbance of working reagent is lower than 1.1 at 334 nm the reagent can not be used.

Assay conditions

Method: kinetic (decreasing)

Working reagent 1 ml Sample 25 µl

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

Two reagent procedure

R1 1 ml Sample 50 µl

Mix and incubate for 1 minute.

R2 1 ml

Mix and after 1 minute incubation, measure the change of absorbance per minute (ΔA/min) for 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 or

Randox Calibration Serum Level II

Calibration frequency

- Two point calibration is recommended
- as required following quality control procedures.

Calculation using calibration

- ΔA_{sample}/ΔA_{standard} x C_{standard} = C_{sample}

A = Absorbance, C = Concentration

α-hydroxybutyrate+NADH+H⁺ → α-hydroxybutyrate+NAD⁺

Reference values

Serum α-HBDH activity: 80-220 U/l (1.33-3.67 µkat/l)

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

Reagents

1. Reagent (R1)

Phosphate buffer, pH=7.50 62 mmol/l α-hydroxybutyrate 6.2 mmol/l

2. Reagent (R2)

NADH 240 µmol/l

Precautions

Discard closely reagent. Avoid contamination by using clean disposable devices (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.

Samples

Serum

PERFORMANCES DATA

Reproducibility

Sample Average activity (U/l) SD CV%

- sample I 153.8 1.29 0.84
- sample II 248.7 1.98 0.79

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=0.9999

Linear regression: y(U/l)= 1.045x-2.623

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

Specificity

Bilirubin 855 µmol/l (50 mg/dl), lipid 200mg/dl, glucose 27.7 mmol/l (500mg/dl) and ascorbic acid 2.84 mmol/l (50mg/dl) don’t interfere with the assay up to the given levels to.

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
- Temperature limitation
- Batch Code
- Code

Bibliography