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# ALPHA-HBDH

STABLE LIQUID



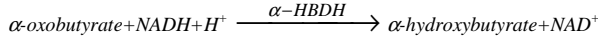
Cat. No.:	45961	45962	45963
	100 ml	600 ml	10x20 ml
	(1x50 ml + 1x50 ml)	(1x300 ml+ 1x300 ml)	(10x10 ml+ 10x10 ml)

## Reagent kit for determination of $\alpha$ -hydroxybutyrate dehydrogenase ( $\alpha$ -HBDH) activity in serum. DGKC method.

There is a significant difference between affinities of lactate dehydrogenase isoenzymes for  $\alpha$ -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  $\alpha$ -oxobutyrate substrate into  $\alpha$ -hydroxybutyrate while  $NAD^+$  is formed. The rate of decrease in absorbance is proportional to the  $\alpha$ -hydroxybutyrate dehydrogenase activity.



### Reference values

**Serum  $\alpha$ -HBDH activity:** 80-220 U/l (1,33-3,67  $\mu$ kat/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

$\alpha$ -oxobutyrate 6.2 mmol/l

#### 2.Reagent (R2)

NADH 240  $\mu$ mol/l

### Precautions

Discard cloudy 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 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  
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)

#### •One reagent procedure

Working reagent	1 ml
Sample	25 $\mu$ l

Mix and after 1 minute incubation, measure the change of absorbance per minute ( $\Delta A/\text{min}$ ) for 3 minutes.

#### •Two reagent procedure

R1	1 ml
Sample	50 $\mu$ l

Mix and incubate for 1 minute.

R2	1 ml
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Mix and after 1 minute incubation, measure the change of absorbance per minute ( $\Delta A/\text{min}$ ) 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

- 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

#### One reagent procedure

340 nm:  $\Delta A/\text{min} \times 7014 = U/l$ ;  $\Delta A/\text{min} \times 116,9 = \mu\text{kat/l}$

334 nm:  $\Delta A/\text{min} \times 7150 = U/l$ ;  $\Delta A/\text{min} \times 119,2 = \mu\text{kat/l}$

#### Two reagent procedure

340 nm:  $\Delta A/\text{min} \times 7478 = U/l$ ;  $\Delta A/\text{min} \times 124,6 = \mu\text{kat/l}$

334 nm:  $\Delta A/\text{min} \times 7616 = U/l$ ;  $\Delta A/\text{min} \times 126,9 = \mu\text{kat/l}$

### 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 1200 U/l (20,0  $\mu$ kat/l)  $\alpha$ -HBDH 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 7.150 U/l (0,12  $\mu$ kat/l)  $\alpha$ -HBDH activity at 334 nm.

### Precision

Sample	Reproducibility		
	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  $\alpha$ -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  $\mu$ mol/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

For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

Batch Code

Code

### Bibliography

Deutsche Gesellschaft Für Klinische Chemie Z. Klin. Chem U.Klin. Biochem. 1972, 10: 182