HBDH

IFCC. Kinetic UV

Quantitative determination of α-hydroxybutyrate dehydrogenase (HBDH)

CE IVD

PACKAGING

Ref.: 101-0462	Cont.: 20 x 3 mL
Ref.: 101-0602	Cont.: 10 x 10 mL

Store at 2-8ºC

CLINICAL SIGNIFICANCE

α-Hydroxybutyrate dehydrogenase (HBDH) is an isoenzyme of lactate dehydrogenase (LDH). Heart muscles contain high concentrations of HBDH. The serum concentration of HBDH is elevated in myocardial damage.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

α-Hydroxybutyrate dehydrogenase (HBDH) catalyses reduction of α -ketobutyrate to α -hydroxybutyrate with concomitant oxidation of NADH to NAD, according to the following reaction:

The rate of NADPH formation, measured photometrically, is proportional to the catalytic concentration of HBDH present in the sample[1].

REAGENTS

R 1 Buffer	TRIS pH 7.2 α -Ketobutyrate	50 mmol/L 3 mmol/L
R 2 Substrate	NADH	0.18 mol/L

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

PREPARATION

Working reagent (WR):

Dissolve (→) one tablet of R 2 Substrate with one vial of R 1.

Cap and mix gently to dissolve contents.

Stability: 24 hours at 2-8º C.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.

Do not use the tablets if appears broken.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm < 1.00.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25º C, 30º C o 37º C (± 0.1º C)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma[1].

Stability: 3 days at 2-8º C. Free of hemolysis.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

1. Assay conditions:
   Wavelength: . . . . .340 nm
   Cuvette: . . . . 1 cm light path
   Constant temperature . . . . .25º C / 30º C / 37º C
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

	25º - 30º C	37º C
WR (mL)	1.0	1.0
Sample (µL)	40	20

4. Mix, incubate for 1 minute.
5. Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1 min intervals thereafter for 3 min.
6. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

CALCULATIONS

25º- 30º C	ΔA/min x 4127* = U/L HBDH	* Tv X 1000	Tv= total volum (mL) ε NADH= 6.22 at 340 nm
37ºC	ΔA/min x 8095* = U/L HBDH		

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Temperature conversion factors

To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25º C	30º C	37º C
25º C	1.00	1.18	1.30
30º C	0.85	1.00	1.09
37º C	0.77	0.92	1.00

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagents and technique for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

25º C	30º C	37º C
< 140 U/L	< 165 U/L	< 182 U/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: Up to linearity limit of 0.150 ΔA/min.

If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl (9 g/L) and multiply the result by 10.

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results of the performance characteristics depend on the analyzer used.

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INTERFERENCES

Haemolysed serum samples should not be used[1].

A list of drugs and other interfering substances with HBDH determination has been reported by Young et. al[2,3].

BIBLIOGRAPHY

1. Rosalki SB. A simple colorimetric method for the determination of -hydroxybutyrate dehydrogenase activity. Clin Path 15:556 (1962).
2. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
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