β-hydroxybutyrate Assay Kit (β-Hb)

Method: β-hydroxybutyrate dehydrogenase

Cat .No.	Size	Instrument
GB128T	R1:1×60 ml R2:1×20 ml	For Hitachi 717 & ShimadzuCL7200/8000
GS129T	R1:1×60 ml R2:1×20 ml	For Hitachi 917 & OlympusAU640/400/600
Calibrator	1×2 ml	

INTENDED USE

For the *in vitro* quantitative determination of D-3-Hydroxybutyrate in serum and plasma.

CLINICAL SIGNIFICANCE[1]

Ketosis is a common feature in acutely ill patients. In subjects suffering from starvation, acute alcohol abuse, or diabetes mellitus ketosis can result in severe life threatening metabolic acidosis. The presence and degree of ketosis can be determined by measuring blood levels of D-3-Hydroxybutyrate. Ordinarily, D-3-Hydroxybutyrate is the ketoacid present in the greatest amount in serum. It accounts for approximately 75% of the ketone bodies which also contain acetoacetate and acetone. During periods of ketosis, D-3-Hydroxybutyrate increases even more that the other two ketoacids, acetoacetate and acetone, and has been shown to be a better index of ketoacidosis including the detection of subclinical ketosis. In diabetics, the measurement of D-3-Hydroxybutyrate as well as the blood glucose is needed for the assessment of the severity of diabetic coma and is essential for the exclusion of hyperosmolar non-ketotic diabetic coma. Moreover, the insulin requirements are often based on the extent of the existing hyperketonemia shown by the blood levels of D-3-Hydroxybutyrate is therefore extremely important.

ASSAY PRINCIPLE[2]

The method is based on the oxidation of D-3-hydroxybutyrate to acetoacetate by the enzyme D-3-Hydroxybutyratedehydrogenase. Concomitant with this oxidation, the cofactor NAD^+ is reduced to NADH and the associated change of absorbance can be directly correlated with the D-3-hydroxybutyrate concentration.

$$\text{D-3-hydroxybutyrate} + NAD^+ \xrightarrow[\text{dehydrogenas}]{\text{D-3-hydroxybutyrate}} \text{acetoacetate} + H^+ + \text{NADH}$$

SAMPLE COLLECTION AND PREPARATION

Serum or plasma samples.
Serum or plasma samples are stable for a week at 2-8°C.

REAGENT COMPOSITION

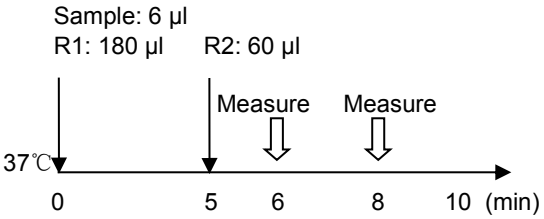
Contents	Concentration of solutions
Reagent 1 (R1)	
Tris buffer	100 mmol/L
D-3-hydroxybutyrate dehydrogenase	2 KU/L
Reagent 2 (R2)	
NAD^+	2.5 mmol/L
Oxalic acid	20 mmol/L

STABILITY AND PREPARATION OF REAGENTS

All reagents are ready to use.
Stable up to the expiry date when stored at 2-8℃.
The reagents are stable for 1 month after opening and kept at 2-8℃.

ASSAY PROCEDURE

Test Procedure for Analyzers(HITACHI 7170/917)
Assay Mode: 2 Point Rate , 20-27
Wave Length (main/sub): 340 nm/700 nm



1. Mix 6 µl sample with 180 µl R1 and incubate at 37℃ for 5 minutes.
2. Add 60 µl R2 into cuvette, mix and incubate for 1 minute at 37℃.
3. Read initial absorbance and start timer simultaneously, read again after 1 and 2 minutes.
4. Calculate absorbance change per minute (ΔA/min).

CALCULATION

$$\text{Concentration} = \frac{\Delta A_{sample}/\text{min}}{\Delta A_{calibrator}/\text{min}} \times \text{Calibrator value}$$

CALIBRATION

Recommend that this assay should be calibrated using Gcell Calibrator (Cat .No. GC-D-3-HB).

QUALITY CONTROL

Randox Assayed Multi-sera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check reaction temperature.
3. Check expiration date of kit and contents.

REFERENCE VALUE

Plasma fasting levels: 0.02 - 0.27 mmol/L.
It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

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SPECIFIC PERFORMANCE CHARACTERISTICS

LINEARITY
The method is linear up to 4.5 mmol/L. Samples with higher concentrations should be diluted 1+1 with redistilled water and reassayed. Multiply the result by 2.

PRECISION
The CV of the test should be CV ≤5%.

Intra assay precision		
N=20	Level1	Level 2
Mean (mmol/L)	0.28	1.11
SD	0.005	0.010
Cv	1.87%	0.93%
Inter assay precision		
N=5	Level1	Level 2
Mean (mmol/L)	0.35	1.22
SD	0.007	0.016
Cv	2.09%	1.31%

SENSITIVITY
The minimum detectable level that can be distinguished from zero has been determined as 0.04 mmol/L.

INTERFERENCE
The following analytes were tested up to the levels indicated and found not to interfere:
Hemoglobin: 400 mg/dl
Intralipid: 600 mg/dl
Bilirubin: 50 mg/dl
Ascorbic acid: 50 mg/dl

CORRELATION
This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:
Y=0.994x-0.012, R2=0.993, 70 patient samples were analyzed .

SAFETY PRECAUTIONS AND WARNINGS
1. For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handing laboratory reagents.
2. Reagents contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
3. Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.
4. All specimens used in this test should be considered potentially infectious. Universal Precautions, as they apply at your facility, should be used for handling and disposing of materials during and after testing.

REFERENCES
1. Mcmurray,C.H., Blanchflower, W.J., Rice, D.A., ClinChem., 1984;30:No.3.
2. Li, P.K., Lee, S.T. Macgillvray, M.H., et al. ClinChem. 1980; 26:1713-1717

INDEX OF SYMBOLS

	Manufacture
REF	Catalogue Number
LOT	Lot number
	Date of manufacture
	Use by(Expiration date)
IVD	For In-Vitro Diagnostic use only
2℃ 8℃	Stored at 2-8℃
	Attention:See instruction for use
EC REP	Authorized Representative in the European Company

Manufacture：Beijing Strong Biotechnology, Inc.
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