Pariksha Biotech
A game changer in IVD

Serum Ketone Estimation Kit

High-Q D-3-Hydroxybutyrate (β-hydroxybutyrate) (Enzymatic Method)

HIGH®
Command on Quality

Intended Use: Kit for the quantitative in vitro determination of D-3-Hydroxybutyrate (β-HBDH or D3H) in human serum on photometric systems

Clinical Significance:

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 β-hydroxybutyrate. Ordinarily β-hydroxybutyrate is the ketoacid present in the greatest amount in serum. It accounts for approximately 75% of the ketone bodies that also contain acetoacetate and acetone. During periods of ketosis, β-hydroxybutyrate increases even more than 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 β-hydroxybutyrate as well as blood glucose is needed for the assessment of the severity of diabetic coma and is essential for the exclusion of hyper osmolar non-ketotic diabetic coma. Moreover, the insulin requirements are often based on the extent of the existing hyper ketonemia shown by the blood levels of β-hydroxybutyrate. A specific enzymatic assay for β-hydroxybutyrate is therefore extremely important in the assessment of ketosis.

Principle:

1. D-3-Hydroxybutyrate + NAD ---(D-3-Hydroxybutyrate dehydrogenase)---> Acetoacetate + NADH + H^+
2. NADH+INT(oxidized) ---(Diaphorase)---> NAD + INT (reduced)

D3-Hydroxybutyrate (D-3-hydroxybutyrate) in the presence of NAD gets converted to acetoacetate and NADH by D3-hydroxybutyrate dehydrogenase (D-3-hydroxybutyrate dehydrogenase). The NADH produced reacts with INT in the presence of diaphorase to produce color at 505 nm. The absorbance difference at 505/630 nm is proportional to the β-hydroxybutyrate concentration in the sample.

Reagent Composition:

Components and Concentrations

R1: Buffer pH 8.5 < 150 mmol/L
β-Hydroxybutyrate-dehydrogenase ≥ 1 kU/L

R2: Buffer pH 4.3 < 70 mmol/L
NAD < 25 mmol/L

Calibrator: β-Hydroxybutyrate (Conc: Lot Specific)

Storage and Stability:

All the reagents are to be stored at 2-8^{0}C and are stable till the expiry date mentioned on the labels when properly stored.

Specimen preparation:

Un hemolysed Serum.
Stability of specimen: 1 week at 2-8 °C

Assay Procedure:

System Parameters

Reaction Type (Mode)	End Point
Reaction Direction	Increasing
Wave Length	505 nm /630 nm (Bichromatic)
Flow Cell Temp.	37°C
Blank	Reagent Blank
Reagent Volume	400 µl (R1) + 100 µl (R2)
Sample	10 µl
Calibrator Concentration	Lot Specific (Check the Labels)
Linearity	50 mg/dl
Low Normal	0.1 mg/dL
High Normal	3.0 mg/dL

Reagent	Blank	Calibrator	Test
D3H R1	400 µl	400 µl	400 µl
Calibrator	----	10 µl	----
Sample	----	----	10 µl
D3H R2	100 µl	100 µl	100 µl

Mix well incubate for 10 minutes at 37°C

Read absorbance of Calibrator (C) and Test (T) against Reagent Blank (B) at 505/630 nm bichromatically

(If the end user does not have two filters in the analyzer he can perform the assay at 505 nms alone monochromatically)

Calculations:

$$\text{D3H Concentration in mg/dL} = \frac{\text{Abs. of Test}}{\text{Abs. of Calibrator}} \times \text{D3H Calibrator Concentration (Lot Specific)}$$

Reference Range:

0.10 - 3.00 mg/dL

It is recommended that each laboratory should establish its own reference interval.

Quality Control:

Pariksha's D3H Controls are recommended for daily quality control.The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calibration:

The assay requires the use of a D3-hydroxybutyrate calibrator. Recalibration is recommended at anytime if one of the following events occurs:

- The Lot number of reagents changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

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Performance characteristics:

Linearity:

The method is linear up to 50.0 mg/dL
Samples above this concentration should be diluted 1+1 with 0.9% NaCl solution and the result multiplied by 2.

Interference:

The effect of the following substances can be neglected if the concentrations are at or below the given values.

Substances	Concentrations
Glucose	1000 mg/dl
Acetoacetic acid	5 mmol/L
Creatinine	5 mg/dl
Ascorbic	3 mg/dl
Bilirubin	10 mg/dl
Uric Acid	15mg/dl
Triglycerides	400mg/dl
Cholesterol	300 mg/dl
Lactate dehydrogenase	1200 U/ml
Sodium Lactate	90mg/dl

References:

1. Foster DW and McGarry, N Eng J Med 309, 159(1983).
2. Persson B, Scand J Clin Lab Invest 25, 9(1969).
3. Wildenhoff KE, Clin Chem 25,475(1978).
4. Koch DD and Feldbruegge DH, Clin Chem 33(10), 1761(1987).
5. Li PK, Lee JT, MacGillivray MH, Schaefer PA and Siegel JH, Clin Chem 26(12), 1713(1980).
6. Stephens JM, Suway MJ and Watkins PJ, Diabetes 20(7), 485(1971).
7. Harano Y, Kosugi K, Hyosu T, Uno S, Ichikawa Y and Shigeta Y, Clin Chem Acta 134, 327(1983).
8. MacGillivray MH, Li PK, Lee JT and et al., J Clin Endocrinol Metab 54, 665, (1982).

Ordering Information:

Ref./Cat. No.	Pack Size	Presentation
P-D3H - 25	25 ml	Two Liquid Reagents with Calibrator
P-D3H - 50	2 x 25 ml	

Product Features:

- **Liquid Stable, Ready to use Two Reagents**
- **10 Minutes End Point Assay**
- **Linearity : 50.0 mg/dL**
- **Bichromatic Estimation**
- **Measuring Wavelength : 505/630 nm**
- **Serum as preferred Specimen**
- **Available as multipurpose reagents and dedicated system packs**

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

eIFU Indicator

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Manufactured in India by :
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AN ISO 13485 Certified Company

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