BETA-HYDROXYBUTYRATE REAGENT KIT
MD-101108

Intended Use
For the in vitro quantitative measurement of Beta-Hydroxybutyrate (BHB) in urine.
NOT FOR USE IN UNPROFESSIONAL SETTINGS.

Summary and Principle
Out of the three ketone bodies - BHB, acetoacetate and acetone - which are formed in the liver during increased fat breakdown, BHB is the most abundant ketone (~75–80% of total ketones) in ketoacidosis. BHB is a key marker in detecting the severity of diabetic ketoacidosis. Reports have shown that BHB is a better indicator than acetoacetate and acetone for monitoring treatment of diabetic ketoacidosis or even for detecting sub clinical ketosis. Also, accurate laboratory measurement of acetoacetate and acetone is difficult because of their inherent instability, whereas BHB is comparatively stable.[1-4]
The following procedure is based on the enzymatic procedure described by David D. Koch and Donald H. Feldbruegge. In this procedure Beta-Hydroxybutyrate dehydrogenase catalyzes the reaction where BHB is converted to acetoacetate with concomitant reduction of NAD to NADH. The increase in absorbance is monitored at 340 nm. The delta absorbance produced is directly proportional to the concentration of BHB in the urine sample.

Reagents
Reagents are packaged ready for use. No preparation is required. Upon opening containers, reagents are stable at 2-8°C for 60 days.
Reagent 1 contains: Buffer, 4.0 mmol Magnesium Chloride, 10 mmol Oxalic Acid, ≥5KU Beta-Hydroxybutyrate dehydrogenase, stabilizer and nonreactive ingredients.
Reagent 2 contains: Buffer, 5mmol NAD, stabilizer and nonreactive ingredients.

Reagents Stability and Storage
Store Reagent 1 and Reagent 2 at 2-8°C. When stored as directed, these reagents are stable until expiration date stated on the label. Reagent 1 and Reagent 2 have been tested to reflect shipping conditions and is stable for the lifespan of the product if frozen up to 5 times or reaching temperatures up to 40°C for up to one week.

Cautions
Handle these reagents using good laboratory practice. DO NOT PIPETTE REAGENT BY MOUTH. Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Instrumentation
MISSION BHB Kit is for use on Mission Diagnostics Affirm C200 [1]and Beckman AU680 Analyzers. Refer to instrument procedure instructions in the instrument manual provided with the specific analyzer.

Specimen Collection and Handling
Use a clean, dry, sterile plastic container with a tightly sealed lid. Test within 2 hours of collection.
Protect from light and heat: Ketones, including BHB, degrade with exposure. Refrigeration is essential if there is any delay in testing. Samples frozen at -20°C are stable for several days.

Quality Control
Store and handle reagents properly before and during use. Every laboratory should establish its own test requirements using controls and calibrators. MISSION Diagnostics provides Calibrators to meet your program needs and which conform to NLCP Guidelines[5]:

MD-101208 – Beta-hydroxybutyrate Calibrator

Specificity, Limitations, and Interferences
Improper storage leads to degradation of BHB. Bacterial contamination: bacteria consume ketones, lowering results. Light exposure degrades ketone bodies. pH extremes (very acidic or alkaline urine) may alter enzymatic activity.

Typical Performance Characteristics
The following performance data was obtained using the Affirm C200 and Beckman AU680 Analyzers. Other instruments may yield different performance data.

Linearity
The following results were obtained on an Affirm C200 and Beckman AU680 Analyzers using the MISSION BHB Reagent Kit on samples containing 1.56, 3.12, 6.25, 12.5 and 25 mg/dL BHB. The table below includes mean, standard deviation (SD) and Coefficient of Variation (CV) for each value.

Mean (mg/dL)	SD	CV%
1.682	0.036	2.1
3.310	0.016	0.5
6.522	0.062	0.9
12.760	0.091	0.7
24.276	0.203	0.8

[1] Also known as Zybio EXC200 Analyzer

Distributed by: Mission Diagnostics LLC, 1 Burton Dr., Meridith, NH 03246
Manufactured by: Diamond Diagnostics Inc., 333 Fiske St., Holliston, MA 01746
Technical Assistance Call: 1.508.429.0450
Email: support@diamonddiagnostics.com

Precision

Studies performed on Affirm C200 and Beckman AU680 Analyzers. The precision of the assay was evaluated following a modification of NCCLS protocol EPT-T2. The within-run precision data was obtained by running two samples in replicates of 20 on the same day. The run-to-run data was obtained by running two samples in replicates over a five-day period.

Within-Run			Run-to-Run		
Mean (mg/dL)	**SD**	**CV%**	**Mean** (mg/dL)	**SD**	**CV%**
8.088	0.031	0.4	8.168	0.189	2.3
24.500	0.130	0.5	25.133	0.598	2.4

Analytical Specificity

Cross contamination studies have not been performed on Affirm C200 and Beckman AU680 Analyzers. Certain reagent/ instrument combinations used in sequence with this assay may interfere with reagent performance and test results. The existence of, or effects of, any potential cross contamination issues are unknown.

Test Conditions

For the data presented in this insert, studies using this reagent were performed on Affirm C200 and Beckman AU680 Analyzers using the parameters listed below.

Limitations

A sample with a BHB concentration exceeding the analytical measuring range should be diluted with deionized water and reanalyzed incorporating the dilution factor in the calculation of the value.

Calibration

Calibration material should be used to calibrate the procedure. The frequency of calibration using an automated system is dependent on the system and the parameters used. If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

Method Parameters

Analyzer Specific Settings

Method type:	Endpoint
Slope:	positive
Units	mg/dL
DOM wavelength	340
SUB wavelength	405
Sample volume	3
R1 volume	150
R2 volume	30
Blank rxn read (cycles)	16 - 20
Sample rxn read (cycles)	36 - 40

Calibration Settings

Calibration Type	Linear
Reagent Blank required	No
Calibrator 1	0
Calibrator 2	3
Calibrator 3	15

Analytical Measuring Range (AMR)

Range: (low)	0
Range: (high)	25

BIBLIOGRAPHY

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