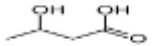
β-Hydroxybutyrate (β-HB) Assay Kit

(Catalog #K632-100; 100 assays; Store at -20˚C)

I. Introduction:

Diabetic ketoacidosis occurs when circulating insulin levels drop to very low levels, shutting off the supply of glucose to the body. The physiological response is for the liver to produce ketone bodies (acetoacetate, acetone, and primarily β-hydroxybutyrate) from the acetyl CoA produced from fatty acid oxidation. The very high rate of ketone body production outstrips the body's ability to utilize them as an energy source and the blood concentration builds up. As rather strong acids, they result in a significant drop in blood pH. BioVision's β-HB Assay kit utilizes β-HB Dehydrogenase to generate a product which reacts with our colorimetric probe with an absorbance band at 450 nm. The kit is an easy and convenient assay to measure β-HB levels in biological samples. The assay is linear for 1-20 nmol β-HB in up to 100 µl samples or 0.01-0.2 mM of β-HB samples.



β-Hydroxybutyric Acid

II. Kit Contents:

Components	K632-100	Cap Code	Part Number
β-HB Assay Buffer	25 ml	WM	K632-100-1
β-HB Enzyme Mix	lyophilized	Green	K632-100-2
β-HB Substrate Mix	lyophilized	Red	K632-100-3
β-HB Standard (1.0 µmol)	lyophilized	Yellow	K632-100-4

III. Storage and Handling:

Store kit at –20˚C, protect from light and moisture. Warm up β-HB Assay Buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

IV. Reagent Preparation and Storage Conditions:

Enzyme Mix: Dissolve with 220 µl β-HB Assay Buffer. Pipette gently to dissolve. Keep on ice. Store at –20˚C. Stable for at least two months

Substrate Mix: Dissolve with 220µl of Assay Buffer before use. Mix well, store at –20˚C, protect from light.

β-HB Standard: Dissolve in 100 µl dH_2O to generate a 10 mM solution. Store at -20°C.

V. β-HB Assay Protocol:

1. **Standard Curve Preparations:** Dilute the β-HB Standard to 1.0 mM by adding 10 µl of the Standard to 90 µl of distilled water, mix well. Add 0, 4, 8, 12, 16, 20 µl to a series of wells. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 4, 8, 12, 16 and 20 nmol per well of the β-HB Standard.
2. **Sample Preparation:** β-HB concentrations can vary over a wide range from normal range: 20 µM-1 mM to diabetic range: 3-5 mM in serum and 10 times that in urine during diabetic ketoacidosis. Due to the presence of interfering substances in blood and urine up to about 5 µl equivalent of such samples can be tested directly. Add samples to test wells. Adjust the volume to 50 µl with β-HB Assay Buffer

   To remove interfering substance from serum, serum sample can be spun filtered (10kDa MWCO spin filter - BioVision cat #1997-25). Filtered serum can be used directly in the assay at 50 µl or up to100 µl per well. Do not use assay buffer in this case. Add enzyme mix and substrate mix as described below. For unknown samples, it may be necessary to test several different doses to ensure the readings are within the range of the standard curve.
3. **Development:**
   Mix enough reagents for the number of samples and standards to be performed: For each well, prepare a total 50 µl Reaction Mix.

β-HB Assay Buffer	46 µl
β-HB Enzyme Mix*	2 µl
β-HB Substrate Mix	2 µl

   Mix and add 50 µl of the Reaction Mix to each well containing β-HB Standard or samples.

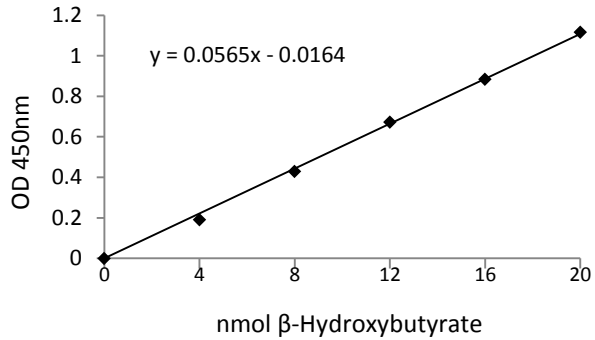
   ***Note:** Reduced pyridine nucleotides NAD(P)H can interfere with the assay. If the presence of these compounds is suspected in the sample, run a background control substituting the 2 µl Enzyme Mix with 2 µl Assay Buffer. The background reading should be subtracted from β-HB sample reading.
4. Incubate at room temperature for 30 min, protect from light.
5. Measure OD at 450 nm.
6. **Calculation:** Correct background by subtracting the 0 β-HB control from all standard and sample readings (Note: The background can be significant and must be subtracted). Plot standard curve nmol/well vs. standard readings. Apply sample readings to the standard curve to get the amount of β-HB in the sample wells.

   The β-HB concentration in the test samples:

   C = Ay/Sv (nmol/µl; or µmol/ml; or mM)

   Where: Ay is the amount of β-HB (nmol) in your sample from the standard curve.
   Sv is the sample volume (µl) added to the sample well.

   β-Hydroxybutyric acid molecular weight: 104.1



β-HB Standard Curve: Assays were performed following the kit protocol.

RELATED PRODUCTS:

Fatty Acid Assay Kit	Triglyceride Assay Kit
Glucose Assay Kit	Cholesterol, LDL/HDL Assay Kits
Glutathione Assay Kits	Ethanol and Uric Acid Assay Kit
NAD/NADH and NADP/NADPH Assay Kits	Lactate Assay Kits
TAC Total Antioxidant Capacity Kit	Pyruvate Assay Kit
Nitric Oxide Detection Kits	Glycogen Assay Kit
Creatinine and Creatine Assay Kits	Glutamate Assay Kit

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		