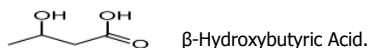


# β-Hydroxybutyrate (β-HB) Assay Kit

(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.



## 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 220 μl of Assay Buffer before use. Mix well, store at -20°C, protect from light.

**β-HB Standard:** Dissolve in 100 μl dH<sub>2</sub>O 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 (10kd MWCO spin filter - BioVision cat #1997-25). Filtered serum can be used directly in the assay at 50 μl or up to 100 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 minutes, protect from light.

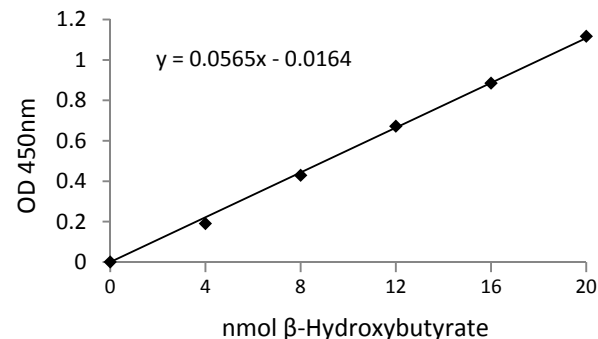
**5.** Measure OD at 450 nm.

**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:

$$C = Ay/Sv \text{ (nmol/}\mu\text{l; or } \mu\text{mol/ml; or mM)}$$

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.

## VI. 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