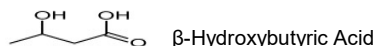


β -Hydroxybutyrate (β -HB) Colorimetric Assay Kit (BN00856)

(Catalog #BN00856; 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. Assay Genie'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 1-10 nmol β -HB in up to 100 μ l samples or 0.01-0.1 mM of β -HB samples.



II. Kit Contents:

Components	BN00856	Cap Code	Part Number
β -HB Assay Buffer	25 ml	WM	BN00856-1
β -HB Enzyme Mix	lyophilized	Green	BN00856-2
β -HB Substrate Mix	lyophilized	Red	BN00856-3
β -HB Standard (1.0 μ mol)	lyophilized	Yellow	BN00856-4

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 μ l 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 μ l of Assay Buffer before use. Mix well, store at -20°C, protect from light.

β -HB Standard: Dissolve in 100 μ l dH₂O 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 μ l of the Standard to 90 μ l of distilled water, mix well. Add 0, 2, 4, 6, 8, 10 μ l to a series of wells. Adjust volume to 50 μ l/well with Assay Buffer to generate 0, 2, 4, 6, 8, and 10 nmol per well of the β -HB Standard.

2. **Sample Preparation:** β -HB concentrations can vary over a wide range from normal range: 20 μ M-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 μ l equivalent of such samples can be tested directly. Add samples to test wells. Adjust the volume to 50 μ l with β -HB Assay Buffer

To remove interfering substance from serum, serum sample can be spun filtered (10kDa MWCO spin filter). Filtered serum can be used directly in the assay at 50 μ l or up to 100 μ l 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 μ l Reaction Mix.

β -HB Assay Buffer	46 μ l
β -HB Enzyme Mix*	2 μ l
β -HB Substrate Mix	2 μ l

Mix and add 50 μ l 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 μ l Enzyme Mix with 2 μ l 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.

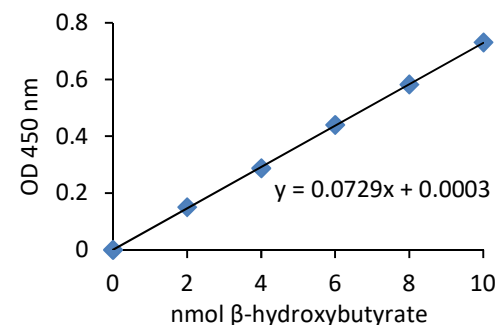
The β -HB concentration in the test samples:

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

Where: Ay is the amount of β -HB (nmol) in your sample from the standard curve.

Sv is the sample volume (μ l) added to the sample well.

β -Hydroxybutyric acid molecular weight: 104.1



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

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
<p>Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</p>		