MA-0132

# β-Hydroxybutyrate Assay

**(100 wells, Colorimetric, OD 450 nm, Store at -20°C)**

## Background Information:

β-hydroxybutyrate (BHB) is a ketone body produced during periods of prolonged fasting, low carbohydrate intake, or intense exercise. A key participant in energy metabolism and fuel utilization, BHB serves as an alternative energy source for various tissues, including brain, heart, and skeletal muscle, when glucose availability is limited. BHB also acts as a signaling molecule, affecting gene expression, oxidative stress, inflammation and other cellular processes. Its metabolic significance extends beyond energy provision, contributing to various physiological and pathological processes. AkrivisBio's β-HB assay is a simple, sensitive method of determining BHB content. In the assay, BHB is oxidized with the formation of NADH which is used to reduce a tetrazolium to a highly colored formazan with absorbance at 450 nm. Assay sensitivity is useful for measuring BHB in samples as low as 10 µM.

## Assay Principle:

1 - β-hydroxybutyrate dehydrogenase oxidizes BHB to acetoacetate forming NADH
2 - NADH transfers an electron to a tetrazolium converting it to a formazan.

## Assay Components:

| | | | |
|---|---|---|---|
| Assay Buffer | 25 ml | WM | MA-0132-A |
| β-Hydroxybutyrate dehydrogenase | lyophil | Green | MA-0132-B |
| NAD/Tetrazolium Mix | lyophil | Red | MA-0132-C |
| β-Hydroxybutyrate Standard | lyophil | Yellow | MA-0132-D |

## Storage and Handling:

Store kit at -20°C prior to use. Warm Assay components to room temperature before use. Centrifuge all small vials for several seconds prior to opening.
**Assay Buffer:** Ready to use as supplied. Store at 4°C
**β-Hydroxybutyrate dehydrogenase**: Dissolve with 220 µl Assay Buffer. Keep on ice while using. Store at -20°C.
**NAD/Tetrazolium Mix:** Dissolve with 220 µl Assay Buffer. Store at -20°C.
**β-HB Standard:** Dissolve in 100 µl DI $H_2O$ giving a 10 mM solution. Store at -20°C.

## Assay Protocol:

**1. Standard Curve:** Transfer 10 µl of the Standard to 90 µl of DI water giving an 0.4 mM working solution. Transfer 0 – 5 – 10 – 15 – 20 - 25 µl to a series of wells in a 96 well plate. Adjust all well volumes to 50 µl with Assay Buffer, giving 0 – 2 – 4 – 6 – 8 – 10 nmol per well.

**2. Sample Preparation:** β-HB content varies widely in body fluids (20 µM - 5 mM in serum) and potentially much higher in urine. Reducing substances in blood and urine can interfere with the assay. They can be minimized by using a 10 kDa spin filter. Add 1 – 50 µl sample to test wells. Adjust all well volumes to 50 µl with Assay Buffer.

Notes:

- Reducing substances and other so-called matrix effects can be dealt with easily. See next page for a method of dealing with significant interference with the assay.
- In rare instances, reduced pyridine nucleotides NAD(P)H are present and interfere with the assay. In this case, run a background control substituting the β-HB Dehydrogenase with 2 µl Assay Buffer. Subtract this background to give a corrected β-HB reading.
- All sample readings must be within the range of the standard curve. If a reading is outside of this range, dilute the sample and rerun.

**3. Initiate Reaction:** Each reaction requires 50 µl of reaction mix. Prepare sufficient reaction mix for the total number of wells to be run:

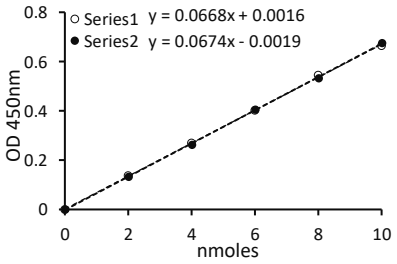
| | **Reaction Mix**: | **Background Control Mix** |
|---|---|---|
| Assay Buffer | 46 µl | 48 µl |
| β-HB Dehydrogenase | 2 µl | ------ |
| NAD/Tetrazolium Mix | 2 µl | 2 µl |

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

**4. Measurement:** Monitor reaction at 450 nm for 30 min. The standards will develop color very quickly, but sample color development can be much slower.

**5. Typical Results:**

| | Standard Raw Values | | Background Corrected Values | |
|---|---|---|---|---|
| nmol Standard | OD | | | |
| 0 | 0.0545 | 0.0555 | 0 | 0 |
| 2 | 0.1903 | 0.1888 | 0.1358 | 0.1333 |
| 4 | 0.3238 | 0.3192 | 0.2693 | 0.2637 |
| 6 | 0.4552 | 0.4598 | 0.4007 | 0.4043 |
| 8 | 0.5977 | 0.5893 | 0.5432 | 0.5338 |
| 10 | 0.7189 | 0.7303 | 0.6644 | 0.6748 |




**6. Calculation:** Subtract the 0 BHB standard reading from all standard readings. Plot the standard curve for the background corrected values. Determine the slope of the standard curve, as this will be used to define BHB amount in unknown samples. Correct unknown samples by subtracting any background controls from their paired samples. Divide the corrected sample readings by the slop of the standard curve to get nmoles of BHB in the sample wells. To correct these values back to BHB content in original samples:

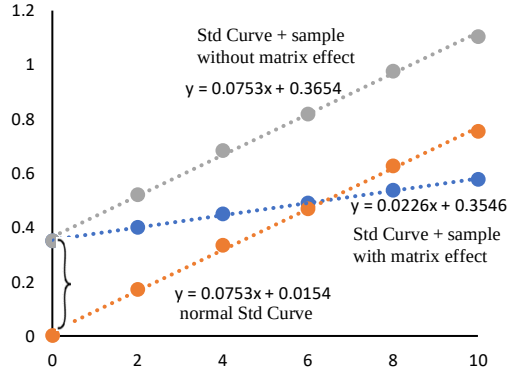
A. nmoles BHB in well / µl sample applied to well = nmoles BHB/µl in sample applied to well

B. nmoles BHB/µl sample applied to well X volume of sample = total nmoles BHB in sample applied to well

C. If sample was diluted due to high signal, multiply by dilution factor.

**Note:** Many metabolites and other chemicals can interfere significantly with a variety of reaction chemistries. For more precise determinations, perform a standard curve in the absence and presence of a constant amount of sample. It's not necessary to run all six standards; use a minimum of 3 (0 – 10 – 20 µl) to verify that the OD response is linear with amount of analyte. The slopes of the two std curves should be the same. The OD offset between them is attributable to the amount of analyte (β-hydroxybutyrate in the current case) in the sample. If the two slopes are different, there is a matrix effect influencing the reaction chemistry. In that case determine the OD difference between the 0 standards and apply the slope of the standard curve run in the presence of sample to it.

Example of a sample with a large matrix effect:




Difference between 0 Standards is 0.35. Slope in the presence of sample is 0.226 OD/nmole.
Amount of analyte in sample with matrix effect = 0.35 OD / 0.226 OD/ nmole = 1.55 nmole

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