Product Manual

β-Hydroxybutyrate (Ketone Body) Assay Kit (Fluorometric)

Catalog Number

MET-5083 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Ketone bodies are water-soluble molecules produced from fatty acid oxidation in the liver and kidney. Acetoacetate, β-hydroxybutyrate (β-HB), and their decarboxylated degradation product, acetone, are the three primary ketone bodies. β-HB is the reduced form of acetoacetate in which a ketone group is converted to an alcohol. β-HB and acetoacetate can be used as an energy source when glucose stores are depleted. Ketone bodies are produced under physiological conditions as fasting, starvation, prolonged and intense exercise, low carbohydrate diets, or untreated type 1 diabetes mellitus. During these instances, gluconeogenesis in the liver produces acetoacetate via two acetyl CoA molecules. β-HB and acetoacetate are transported to peripheral tissues and reconverted into acetyl-CoA for energy via their cells' citric acid cycles. Acetone must be converted in the liver to lactic acid and then pyruvic acid before being converted to acetyl-CoA. It is usually excreted in the urine or exhaled.

High levels of ketone bodies can lead to ketosis, or as in extreme type 1 diabetes, ketoacidosis. Prolonged ketosis may lead to a life-threatening metabolic acidosis. Pathological ketosis may indicate organ failure, hypoglycemia in children, diabetes, alcohol intoxication, corticosteroid or growth hormone insufficiency. The degree of ketosis can be determined by monitoring the blood levels of β-HB.

Ketone bodies are noted for their sweet smell and can be detected in the breath and urine of people with ketosis or ketoacidosis. β-Hydroxybutyrate is the ketoacid most prevalent in serum and accounts for ~75% of all ketone bodies measured. During ketosis, β-HB increases even more than the other ketone bodies and is a more accurate index of ketoacidosis.

Cell Biolabs' β-Hydroxybutyrate Assay Kit is a simple fluorometric assay that measures β-Hydroxybutyrate present in biological samples such as plasma, serum, urine, cell lysates or tissue extracts in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, β-Hydroxybutyrate standards and unknown samples. The total β-Hydroxybutyrate concentrations of unknown samples are determined by comparison with a known β-Hydroxybutyrate standard. The kit has a detection sensitivity limit of approximately ~2 µM β-Hydroxybutyrate.

Assay Principle

Cell Biolabs' β-Hydroxybutyrate Assay Kit is a convenient quantitative tool that measures β-Hydroxybutyrate within biological samples. The assay is based on an enzymatic cycling reaction in which the cofactor NAD^+ is reduced to NADH. NADH reacts with a fluorometric probe that produces a product which can be measured with a standard 96-well fluorometric plate reader. The intensity of the product fluorescence is proportional to the β-Hydroxybutyrate within a sample. Samples and standards are incubated for 30 minutes and then read at λ_{ex} = 530-570 nm/ λ_{em} = 590-600 nm (Figure 1). Samples are compared to a known concentration of β-Hydroxybutyrate standards within the 96-well microtiter plate format.

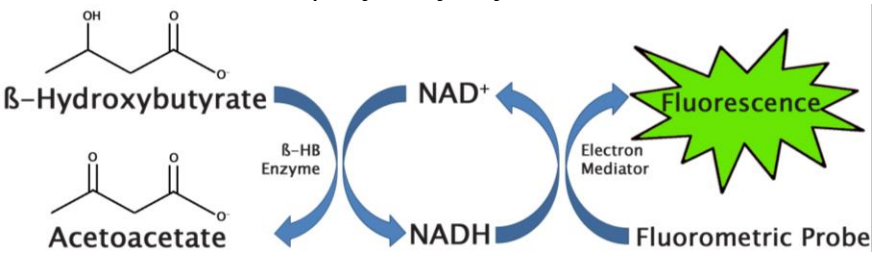

Figure 1. β-Hydroxybutyrate (Ketone Body) Assay Principle.

Related Products

1. MET-5005: Total Bile Acid Assay Kit (Fluorometric)
2. MET-5030: NAD^+/NADH Assay Kit (Fluorometric)
3. STA-390: Total Cholesterol Assay Kit (Fluorometric)
4. STA-619: Free Fatty Acid Assay Kit (Fluorometric)
5. STA-681: Glucose Assay Kit (Fluorometric)

Kit Components (shipped on dry ice)

1. NAD^+ Cofactor (Part No. 50821D): One 0.5 mL amber tube
2. β-HB Enzyme (Part No. 50822D): One 25 µL amber tube
3. β-Hydroxybutyrate Standard (Part No. 50831C): One 50 µL amber tube of a 100 mM solution
4. Assay Buffer (10X) (Part No. 50824A): One 6 mL bottle
5. Fluorometric Probe (40X) (Part No. 50311C): One 150 µL amber tube
6. Electron Mediator (500X) (Part No. 50312C): One 10 µL amber tube

Materials Not Supplied

1. Distilled or deionized water
2. 10 kDa molecular weight cutoff (MWCO) centrifuge spin filter, 0.5mL
3. Standard 96-well fluorescence microtiter plate and/or fluorescence cell culture microplate

Storage

Store the kit at -80°C.

Preparation of Reagents

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity (e.g., Add one 1.5 mL Assay Buffer (10X) tube to 13.5 mL deionized water).
- Reaction Reagent: Just before use, prepare Reaction Reagent for the number of assays being tested by diluting the NAD^+ Cofactor 1:10, β-HB Enzyme 1:200, Fluorometric Probe 1:40, and Electron Mediator 1:500 in 1X Assay Buffer (e.g., for 50 assays, combine 250 µL NAD^+ Cofactor, 12.5 µL β-HB Enzyme, 62.5 µL Fluorometric Probe, and 5 µL Electron Mediator to 2.170 mL of 1X Assay Buffer for a 2.5 mL total solution). Use the Reaction Reagent the same day it is prepared.

Preparation of Samples

These preparation protocols are intended as a guide for preparing unknown samples. The user may need to adjust the sample treatment accordingly. It is highly recommended that all samples should be assayed immediately upon preparation or stored for up to 1 month at -80°C. A trial assay with a representative test sample should be performed to determine the sample compatibility with the dynamic range of the standard curve. High levels of interfering substances may cause variations in results. Samples may be diluted in deionized water as necessary before testing. Run proper controls and account for any sample dilutions. Always run a standard curve with samples.

Notes:

- *Enzymes present in samples may deplete NADH rapidly and affect results. Samples should be deproteinized before using within the assay. A spin filter with a 10 kDa cutoff is recommended for efficient and clean separation.*
- *Avoid samples containing SH groups like DTT, β-mercaptoethanol, or reduced gluathione.*
- *Borate, Methanol (5%), DMSO (5%), Glycerol (5%), and BSA (~1%) may interfere with the assay.*

- Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Remove the plasma layer without disturbing the white buffy layer and store on ice. Filter the solution with a 10kDa spin filter to deproteinate the sample. Collect flow through supernatant and store on ice. Assay immediately or store samples at -80ºC. Plasma samples should be diluted 1:5 to 1:10 with 1X Assay Buffer.
- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer and store on ice. Filter the solution with a 10kDa spin filter to deproteinate the sample. Collect flow through supernatant and store on ice. Assay immediately or store samples at -80ºC. Serum samples should be diluted 1:5 to 1:10 with 1X Assay Buffer.
- Urine: Collect urine in a tube. Samples containing precipitates should be centrifuged at 3000 x g for 10 minutes or filtered to 0.45 µm prior to testing. Assay immediately or store samples at -80ºC. Urine samples should be diluted 1:10 with 1X Assay Buffer.
- Tissue homogenates: Sonicate or homogenize 400 mg tissue sample in 0.5 mL cold 1X Assay Buffer. Centrifuge at 14,000 rpm for 5 minutes at 4°C to remove insoluble material. Filter the solution with a 10kDa spin filter to deproteinate the sample. Collect flow through supernatant and store on ice. Assay immediately or store samples at -80ºC. Tissue samples may be diluted 1:2 with 1X Assay Buffer.
- Cell lysates: Culture cells until confluent and harvest. Avoid using proteolytic enzymes for adherent cells. Use a rubber policeman for these cells. Centrifuge and wash the cell pellet with 1X PBS. Centrifuge to pellet cells and remove wash. Resuspend cells at 1-5 x 1^{06} cells/mL in 0.5 mL cold 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge at 14,000 rpm for 5 minutes 4°C to remove debris. Filter the solution with a 10kDa spin filter to deproteinate the sample. Collect flow through supernatant and store on ice. Assay immediately or store samples at -80ºC. Perform dilutions in cold deionized water or 1X Assay Buffer if needed.

Preparation of Standard Curve

Prepare a series of β-hydroxybutyrate standards according to Table 1. Prepare standards immediately before use. Do not store standard solutions.

Standard Tubes	100 mM β-Hydroxybutyrate Standard (µL)	1X Assay Buffer (µL)	β-Hydroxybutyrate (µM)
1	5 µL Standard Stock	1995	250
2	250 µL of Tube #1	250	125
3	250 µL of Tube #2	250	62.5
4	250 µL of Tube #3	250	31.3
5	250 µL of Tube #4	250	15.6
6	250 µL of Tube #5	250	7.8
7	250 µL of Tube #6	250	3.9
8	250 µL of Tube #7	250	2.0
	0	250	0

Table 1. Preparation of β-Hydroxybutyrate Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50 µL of each β-Hydroxybutyrate standard or unknown sample into wells of a 96-well fluorescence microtiter plate.
3. Add 50 µL of Reaction Reagent to each well. Mix the well contents thoroughly and incubate for 30 minutes at room temperature protected from light.
4. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.
5. Calculate the concentration of β-Hydroxybutyrate within samples by comparing the sample RFU to the standard curve.

Example of Results

The following figures demonstrate typical β-Hydroxybutyrate Assay Kit results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

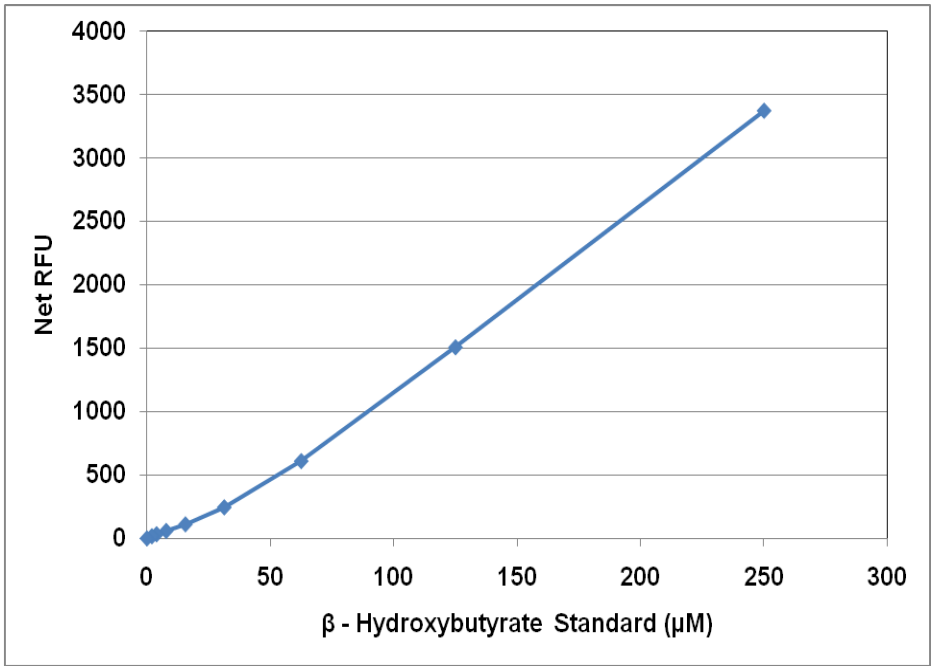

Figure 2: β-Hydroxybutyrate Standard Curve.

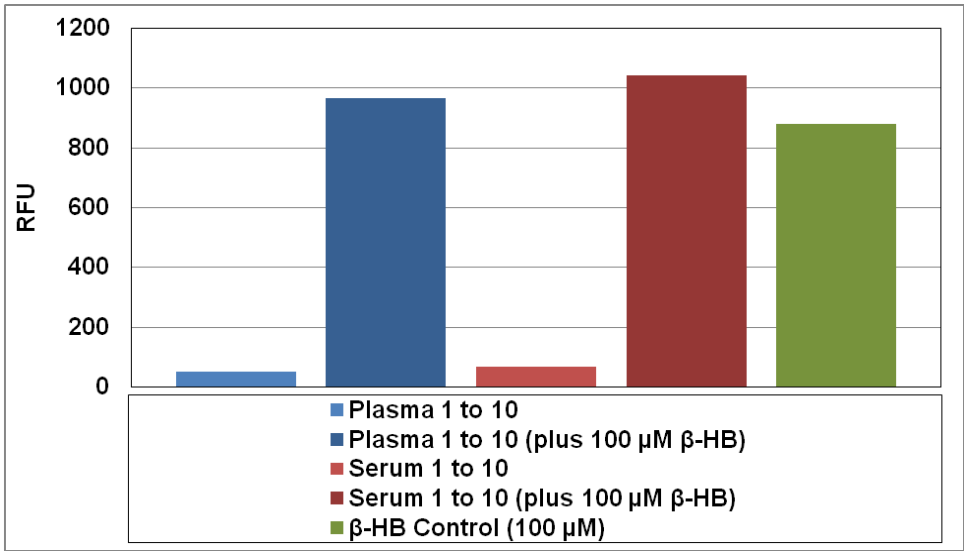

Figure 3: β-Hydroxybutyrate Detection. Normal human plasma and serum were both deproteinated and diluted 1:10 in 1X Assay Buffer prior to testing with and without 100 µM β-Hydroxybutyrate spiked into them. The samples were tested according to the assay protocol. Background values have been subtracted.

Calculation of Results

1. Calculate the average fluorescence values for every standard, control, and sample. Subtract the average zero standard value from itself and all standard and sample values. This is the corrected background fluorescence. If sample background control value is high, subtract the sample background control value from the sample reading.

2. Plot the corrected fluorescence for the β-Hydroxybutyrate standards against the final concentration of the standards from Table 1 to determine the best slope (μM^{-1}). See Figure 2 for an example standard curve.
3. Since all NAD^+ is converted to NADH by the Reaction Reagent, use the standard curve to determine the total β-Hydroxybutyrate concentration within the sample. Determine the total concentration of the samples with the equation obtained from the linear regression analysis of the standard curve. Substitute the corrected fluorescence values for each sample. Remember to account for dilution factors.

$$\textbf{β-Hydroxybutyrate (μM)} = \left[\frac{\textbf{Sample corrected fluorescence}}{\textbf{Slope}} \right] \textbf{x Sample dilution}$$

References

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2. Kashiwaya, Y., et al. (2000) *Proc. Natl. Acad. Sci. USA* **97**: 5440-5444.
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5. Taggart, A.K.P., et al. (2005) *J. Biol. Chem.* **280(29)**: 26649-26652.
6. Veech, R.L. (2004) *Prostaglandins Leukot. Essent, Fatty Acids* **70(3)**: 309-319.

Warranty

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Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com