Amplite™ Fluorimetric Beta-Hydroxybutyrate (Ketone Body) Assay Kit

Catalog number: 13831
Unit size: 200 Tests

Component	Storage	Amount
Component A: Enzyme Mix	Freeze (<-15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)
Component C: NAD	Freeze (<-15 °C), Minimize light exposure	1 vial
Component D: β-Hydroxybutyrate (β-HB) Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (10 µL)

OVERVIEW

Ketone bodies are produced by the liver and used peripherally as an energy source when blood glucose levels drop. The two main ketone bodies are Beta-hydroxybutyrate (Beta-HB) and acetoacetate, while acetone is the third abundant ketone body. Normally these two predominant ketone bodies are present in small amounts in the blood during fasting and prolonged exercise. In patients who have diabetes, alcohol or salicylate poisoning, hormone deficiency, childhood hypoglycemia and other acute disease states, large quantities of ketone bodies are found in the blood. The over-production and accumulation of ketone bodies in the blood (ketosis) can lead to pathological metabolic acidosis (ketoacidosis). In extreme cases, ketoacidosis can be fatal. Blood ketone testing methods that quantify Beta-HB, the predominant ketone body in the blood (approximately 75%) have been used for diagnosing and monitoring treatment of ketoacidosis. Amplite™ Fluorimetric Beta-Hydroxybutyrate Assay Kit offers a sensitive fluorescent assay for measuring Beta-HB levels in biological samples. This assay is based on an enzyme coupled reaction of Beta-HB, in which the product NADH can be specifically monitored by a fluorescent NADH sensor. The fluorescence signal can be measured by a fluorescence microplate reader. With this Fluorimetric Beta-hydroxybutyrate Assay Kit, we were able to detect as low as 1.4 µM Beta-HB in a 100 µL reaction volume.

AT A GLANCE

Protocol summary

1. Prepare β-HB working solution (50 µL)
2. Add β-HB standards or test samples (50 µL)
3. Incubate at room temperature for 10 - 30 min
4. Monitor fluorescence increase at Ex/Em = 540/590 nm

Important To achieve the best results, it's strongly recommended to use the black plates. Thaw one vial of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. NAD stock solution (100X):
Add 100 µL of H_2O into the vial of NAD (Component C) to make 100X NAD stock solution.

2. β-HB standard solution (100 mM):
Add 1 mL of H_2O or 1X PBS buffer into the vial of β-HB standard (Component D) to make 100 mM β-HB standard solution.

PREPARATION OF STANDARD SOLUTION

β-HB standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13831

Add 10 µL of β-HB standard solution (100 mM) into 990 µL 1x PBS buffer to generate 1000 µM β-HB standard solution (HB7). Take the 1000 µM β-HB standard solution and perform 1:3 serial dilutions in 1x PBS to get serial dilutions of β-HB standard (HB6 - HB1).

Note Diluted β-HB standard solution is unstable and should be used within 4 hours.

PREPARATION OF WORKING SOLUTION

1. Add 5 mL of Assay Buffer (Component B) into one bottle of Enzyme Mix (Component A).

2. Add 50 µL NAD stock solution into the bottle of Component A+B, and mix well to make β-HB working solution (Component A+B+C).

Note This β-HB working solution is not stable, use it promptly and avoid direct exposure to light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of β-HB standards and test samples in a clear bottom 96-well microplate. HB = β-HB standard (HB1 - HB7, 1 to 1000 µM); BL = blank control; TS = test sample.

BL	BL	TS	TS
HB1	HB1	...	...
HB2	HB2	...	...
HB3	HB3		
HB4	HB4		
HB5	HB5		
HB6	HB6		
HB7	HB7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
HB1-HB7	50 µL	Serial Dilution (1 to 1000 µM)
BL	50 µL	1X PBS Buffer
TS	50 µL	Test Sample

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

1. Prepare β-HB standards (HB), blank control (BL) and test samples (TS) into a solid black 96-well microplate according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of β-HB working solution to each well of β-HB standard, blank control, and test samples to make the total volume of 100 µL/well. For a 384-well plate, add 25 µL of β-HB working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 10 - 30 minutes, protected from light.

4. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 530 - 570 nm, Emission = 590 - 600 nm (optimal Ex/Em = 540/590 nm, cut off at 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate β-Hydroxybutyrate samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

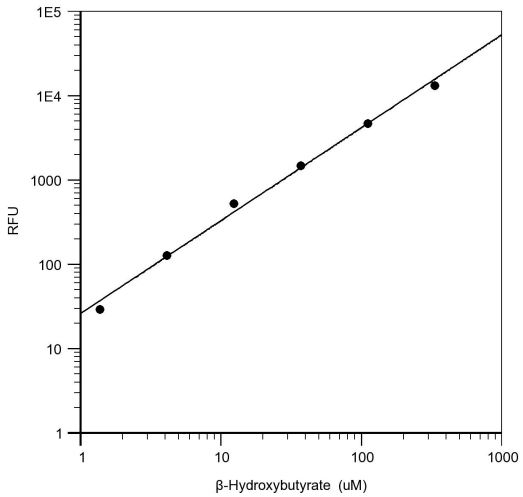

Figure 1. β-Hydroxybutyrate (β-HB) dose response was measured with Amplite™ Fluorimetric β-Hydroxybutyrate Assay Kit on a solid black 96-well plate using a Gemini microplate reader.

DISCLAIMER