Technical Bulletin

High Sensitivity Beta-Hydroxybutyrate (Ketone Body) Assay Kit

Catalogue number MAK547

Product Description

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 (β-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.[1] The over-production and accumulation of ketone bodies in the blood (ketosis) can lead to pathological metabolic acidosis (ketoacidosis).[1] Blood ketone testing methods that quantify β-HB, the predominant ketone body in the blood (approximately 75%) have been used for diagnosing and monitoring treatment of ketoacidosis.[1].

The 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 detection limit of this assay kit is 1.4 µM β-HB in a 100 µL reaction volume.

Components

The kit is sufficient for 200 fluorometric assays in 96-well plates.

- Enzyme Mix — 1 Vial
  Catalogue Number MAK547A
- Assay Buffer — 10 ml
  Catalogue Number MAK547B
- NAD — 1 Vial
  Catalogue Number MAK547C
- β-Hydroxybutyrate (β-HB) Standard — 10 µL
  Catalogue Number MAK547D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Fluorescence multiwell plate reader.
- Black, flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes.
- Phosphate Buffered Saline (Catalogue Number PPB006 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Equilibrate to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Note: All unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Preparation of Stock Solutions

NAD Stock Solution (100X): Add 100 µL of purified water into the vial of NAD to make 100X NAD stock solution.

β-HB Standard Solution (100 mM): Add 1 mL of purified water or 1X PBS buffer into the vial of β-HB standard to make 100 mM β-HB standard solution.

Preparation of β-HB Standard

Add 10 µL of β-HB standard solution (100 mM) into 990 µL 1x PBS buffer to generate 1000 µM β-HB standard solution (HB1).

Using the 1000 µM β-HB standard solution, perform 1:3 serial dilutions in 1x PBS to get serial dilutions of β-HB standard (HB2 – HB7) as per Table 1.

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

Table 1.

Serial dilution of Beta-Hydroxybutyrate (β-HB) Standard.

Dilution	β-HB Std Vol (µL)	Serial Dilution Source	1X PBS Vol (µL)	Conc (µM)
HB1	225	from 1000 µM stock	0	1000
HB2	75	From HB1	150	333.3
HB3	75	From HB2	150	111.1
HB4	75	From HB3	150	37.0
HB5	75	From HB4	150	12.3
HB6	75	From HB5	150	4.1
HB7	75	From HB6	150	1.4

Preparation of β-HB Working Solution

1. Add 5 mL of Assay Buffer into one vial of Enzyme Mix.
2. Then add 50 µL NAD stock solution to the vial and mix well.

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

Assay Reaction

1. Add 50 µL of each β-HB standard, blank (1x PBS), and test samples into a black 96-well microplate.
2. Add 50 µL of β-HB working solution to each well of standard, blank, and test sample to make the total reaction volume of 100 µL/well.
3. Incubate the reaction at room temperature for 10 - 30 minutes, protected from light.

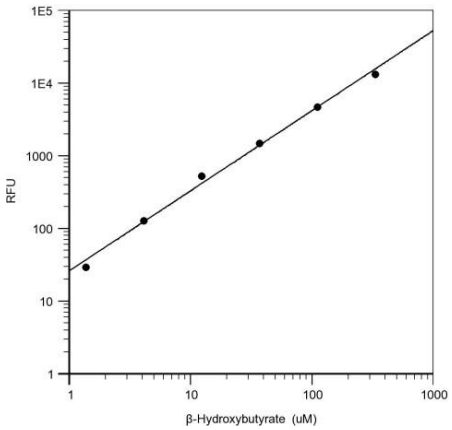
Measurement

1. Read the fluorescence (F) at λ_{Ex} =530- 570nm/λ_{Em} =590-600 nm. (Optimal $\lambda_{Ex}/\lambda_{Em}$ = 540/590 nm, cut off at 570 nm).

Results

1. The reading (RFU) obtained from the blank well is used as a negative control.
2. Subtract the blank value from the standards' readings to obtain the baseline corrected values.
3. Plot the standards to obtain a standard curve and equation.
4. This equation can be used to calculate β-Hydroxybutyrate samples.

Figure 1.
Typical β-Hydroxybutyrate Standard Curve



References

Laffel L., Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. *Diabetes Metab Res Rev*, **15(6),** 412-26, Nov-Dec (1999).

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