

Technical Bulletin

Beta-Hydroxybutyrate (Ketone Body) Assay Kit

Catalogue number MAK540

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 (AcAc), 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 beta-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 colorimetric assay for measuring β -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 NADH sensor. The signal can be measured by an absorbance microplate reader with the OD ratio at the wavelength of 570 nm to 610 nm. The detection limit of this assay kit is 4 μ M beta-hydroxybutyrate in a 100 μ L reaction volume.

Components

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

- | | |
|---|------------|
| • Enzyme Mix
Catalogue Number MAK540A | 1 Vial |
| • Assay Buffer
Catalogue Number MAK540B | 10 ml |
| • NAD
Catalogue Number MAK540C | 1 Vial |
| • β -Hydroxybutyrate (β -HB)
Standard
Catalogue Number MAK540D | 10 μ l |

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (example., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear 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 all components to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Preparation of Stock Solutions

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

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

Preparation of β -HB Standard Solutions

1. Add 10 μ L of 100 mM β -HB standard stock solution into 990 μ L 1X PBS buffer to generate 1000 μ M β -HB standard solution (HB1).
2. Use the 1000 μ M β -HB standard solution (HB1) and perform 1:3 serial dilutions in 1x PBS to get serially diluted β -HB standards (HB2 – HB7) as shown in Table 1

Table 1.
Serial dilution of β -HB) Standard

β -HB Standard	β -HB Std Vol (μ L)	1x PBS	Serial Dilution Source	Conc (μ M)
HB1	225	0	1000 μ M stock	1000
HB2	75	150	From HB1	333.33
HB3	75	150	From HB2	111.11
HB4	75	150	From HB3	37.0
HB5	75	150	From HB4	12.3
HB6	75	150	From HB5	4.1
HB7	75	150	From HB6	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 μ L 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 μ L of each β -HB standard, blank (1x PBS), and test samples into a black 96-well microplate.
2. Add 50 μ L of β -HB working solution to each well of standard, blank, and test sample to make the total reaction volume of 100 μ L/well.
3. Incubate the reaction at room temperature for 10 - 30 minutes, protected from light.

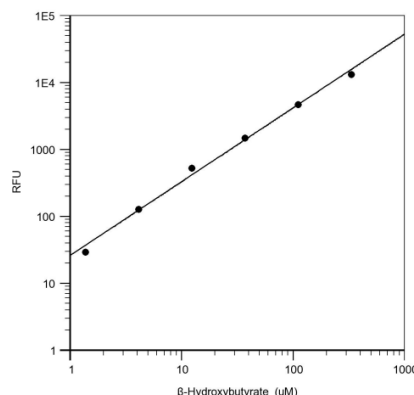
Measurement

Monitor the absorbance increase with an absorbance plate reader at OD ratio of 570/610 nm.

Results

1. The reading (Absorbance) obtained from the blank well is used as a negative control.
2. Subtract the blank value from the standards' readings to obtain the base-line corrected values.
3. Plot the standards readings to obtain the standard curve and equation.
4. The concentration of Beta-Hydroxybutyrate present in the samples may be determined from the standard curve.

Figure 1.
Typical β -Hydroxybutyrate Standard Curve



Reference

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).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

MilliporeSigma, and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.
© 2022 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

mak540pis Rev 11/23

**MILLIPORE
SIGMA**