

Product Information

Beta-Hydroxybutyrate Assay Kit

Catalog Number **MAK041**

Storage Temperature -20°C

TECHNICAL BULLETIN

Product Description

Beta-Hydroxybutyrate, also known as 3-hydroxybutyrate, is a ketone body primarily synthesized in the liver from the oxidation of fatty acids. Ketone body formation, ketogenesis, is elevated when blood glucose levels drop. Moderately elevated blood ketone bodies occur normally during fasting and prolonged exercise. During alcoholic or diabetic ketoacidosis, blood levels of β -hydroxybutyrate can rise to abnormally high levels. β -hydroxybutyrate can be used as a marker for hepatic energy metabolism.

In this assay, β -Hydroxybutyrate concentration is determined by a coupled enzyme reaction, which results in a colorimetric (450 nm) product, proportional to the β -Hydroxybutyrate present.

Components

The kit is sufficient for 100 assays in 96 well plates.

Beta-Hydroxybutyrate Assay Buffer Catalog Number MAK041A	25 mL
Beta-Hydroxybutyrate Enzyme Mix Catalog Number MAK041B	1 vL
Beta-Hydroxybutyrate Substrate Mix Catalog Number MAK041C	1 vL
Beta-Hydroxybutyrate Standard, 1 μmole Catalog Number MAK041D	1 vL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is 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.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Beta-Hydroxybutyrate Assay Buffer – Allow buffer to come to room temperature before use.

Beta-Hydroxybutyrate Enzyme Mix and Beta-Hydroxybutyrate Substrate Mix – Reconstitute with 220 μL of Beta-Hydroxybutyrate Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at -20°C . Use within 2 months of reconstitution and keep cold while in use.

Beta-Hydroxybutyrate Standard – Reconstitute with 100 μL of water to generate a 10 mM solution. Mix well by pipetting, then aliquot and store, protected from light, at -20°C .

Storage/Stability

The kit is shipped on wet ice. Storage at -20°C , protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

β -Hydroxybutyrate Standards for Colorimetric Detection

Dilute 10 μL of the 10 mM (10 nmole/ μL) Beta-Hydroxybutyrate Standard Solution with 90 μL of Beta-Hydroxybutyrate Assay Buffer to prepare a 1 mM (1 nmole/ μL) standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 1 mM Beta-Hydroxybutyrate standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Beta-Hydroxybutyrate Assay Buffer to each well to bring the volume to 50 μL .

Sample Preparation

Tissue (10 mg) or cells (2×10^6) should be rapidly homogenized in 4 volumes of cold β -Hydroxybutyrate Assay buffer. Centrifuge at $13,000 \times g$ for 10 minutes at 4 °C to remove insoluble material.

Serum and other liquid samples can be directly added to the wells.

Note: Samples may be deproteinized with a 10 kDa MWCO spin filter prior to addition to the reaction. This step may be necessary if interfering substances are present in the samples. This step may be necessary when analyzing blood or urine-derived samples.

Bring samples to a final volume of 50 μL with Beta-Hydroxybutyrate Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Note: NADPH from cell or tissue extracts generates background for this assay. To remove the effect of NADPH background, a blank sample may be set up by omitting the Beta-Hydroxybutyrate Enzyme Mix. The blank readings can then be subtracted from the sample and standard readings.

Assay Reaction

1. Set up the Reaction Mixes according to the scheme in Table 1. 50 μL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Blank
Beta-Hydroxybutyrate Assay Buffer	46 μL	48 μL
Beta-Hydroxybutyrate Enzyme Mix	2 μL	—
Beta-Hydroxybutyrate Substrate Mix	2 μL	2 μL

2. Add 50 μL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.
3. Measure the absorbance at 450 nm (A_{450}).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Beta-Hydroxybutyrate Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate β -Hydroxybutyrate standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of Beta-Hydroxybutyrate present in the sample from the standard curve.

Concentration of Beta-Hydroxybutyrate

$$S_a/S_v = C$$

S_a = Amount of Beta-Hydroxybutyrate in unknown sample (nmole) from standard curve

S_v = Sample volume (μ L) added into the wells

C = Concentration of Beta-Hydroxybutyrate in sample

Beta-Hydroxybutyrate molecular weight: 104.1 g/mole

Sample Calculation

Amount of Beta-Hydroxybutyrate (S_a) = 5.84 nmole
(from standard curve)

Sample volume (S_v) = 50 μ L

Concentration of Beta-Hydroxybutyrate in sample

$$5.84 \text{ nmole}/50 \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 104.1 \text{ ng/nmole} = 12.16 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

MF,LS,MAM 10/15-1