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Product Information

High Sensitivity β-Hydroxybutyrate Assay Kit

Catalog Number **MAK272**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

β-Hydroxybutyrate (β-HB), 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.

The High Sensitivity β-Hydroxybutyrate Assay Kit is a simple, sensitive, high-throughput adaptable method for quantifying β-HB when sample quantities are limited or subketogenic levels are suspected. In this assay, β-HB is enzymatically oxidized generating a fluorometric signal (λ_{ex} = 535 nm/λ_{em} = 587 nm), proportional to the amount of β-HB present. The assay is sensitive to 4 μM of β-HB in a variety of biological samples.

This kit is suitable for use with serum, plasma, urine, and other biological fluids, as well as food products such as milk.

Components

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

β-HB Assay Buffer Catalog Number MAK272A	25 mL
High Sensitivity β-HB Probe, in DMSO Catalog Number MAK272B	0.4 mL
β-HB Enzyme Mix Catalog Number MAK272C	1 vl
β-HB Substrate Mix Catalog Number MAK272D	1 vl
β-HB Standard Catalog Number MAK272E	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate. It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence 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.

β-HB Assay Buffer – Allow buffer to come to room temperature before use.

High Sensitivity β-HB Probe – Warm to room temperature to thaw the solution prior to use. Aliquot and store at –20 °C, protected from light. Use within 2 months. Upon thawing, the β-HB Probe is ready-to-use as supplied.

β-HB Enzyme Mix – Reconstitute with 220 μL of β-HB Assay Buffer. Mix well by pipetting (do not vortex). Aliquot and store at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

β-HB Substrate Mix – Reconstitute with 220 μL of β-HB Assay Buffer. Mix well by pipetting (do not vortex). Aliquot and store at –20 °C, protected from light. Use within 2 months of reconstitution.

β-HB Standard – Reconstitute with 100 μL of water to generate a 10 mM (100 nmole/μL) standard solution. Store at –20 °C. Use within 2 months of reconstitution.

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. Use ultrapure water for the preparation of standards.

β-HB Standards for Fluorometric Detection
Dilute 10 µL of the 10 mM β-HB Standard with 990 µL of water to prepare a 0.1 mM (1 nmole/µL) standard solution. Add 0, 1, 2, 3, 4, and 5 µL of the 0.1 mM β-HB standard solution into a 96 well plate, generating 0 (blank), 100, 200, 300, 400, and 500 pmole/well standards. Add β-HB Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
The fluorometric assay requires 50 µL of sample for each reaction (well).

Samples should be deprotienized by filtering with a 10 kDa MWCO spin filter prior to addition to the reaction

Add 2–25 µL samples into wells of a 96 well plate. Bring samples to a final volume of 50 µL with β-HB Assay Buffer.

Notes: For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the range of the standard curve. β-HB concentrations can vary over a wide range. In serum, normal range is 0.02–0.4 mM, but can exceed up to 3 mM in diabetic ketoacidosis and up to 47 mM in alcoholic ketoacidosis.

For samples having high background, prepare parallel sample well(s) as sample background control(s).

Endogenous compounds may interfere with the assay. To ensure accurate determination of β-HB in samples or for samples having low concentration of β-HB, spike samples with a known amount of β-HB Standard (400 pmole).

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

Table 1.
Master Reaction Mix

Reagent	Samples and Standards	Sample Control
β-HB Assay Buffer	42 µL	44 µL
β-HB Probe	4 µL	4 µL
β-HB Enzyme Mix	2 µL	–
β-HB Substrate Mix	2 µL	2 µL

2. Add 50 µL of the Master Reaction Mix to each sample and standard control well. If using a sample background control, add 50 µL of Sample Control Mix to sample control wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate for 30 minutes at room temperature, protected from light.

4. Measure fluorescence intensity (λ_{ex} = 535/ λ_{em} = 587 nm).

Results

Calculations

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

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

Subtract the Sample Blank value from the sample reading to obtain the corrected fluorescence measurement. Using the corrected measurement, determine the amount of β-HB present in the sample from the standard curve.

For spiked samples, correct for interference by using the following equation:

$$\frac{RFU_{sample\ (corrected)}}{(RFU_{sample+\beta\text{-}HB\ Std\ (corrected)}) - (RFU_{sample\ (corrected)})} * \beta - HB\ spike\ (pmol)$$

Concentration of β-HB

$$S_a/S_v = C$$

S_a = Amount of β-HB in the unknown sample (pmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of β-HB in sample

Sample Calculation

Amount of β-HB (S_a) = 258.4 pmole
(from standard curve)

Sample volume (S_v) = 25 mL

Concentration of β-HB in sample:

258.4 pmole/25 μL = 10.34 pmole/μL

10.34 pmole/μL × 342.3 pg/pmole = 3539 pg/μL

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	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 fluorescence assays, use black plates with clear bottoms.
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 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 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 Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	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

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