ab180876

Picoprobe beta Hydrobutyrate (beta HB) Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of β-Hydroxybutyrate in various samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 3b, Last Updated 21 November 2023

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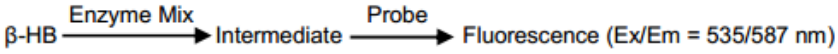
1. Overview

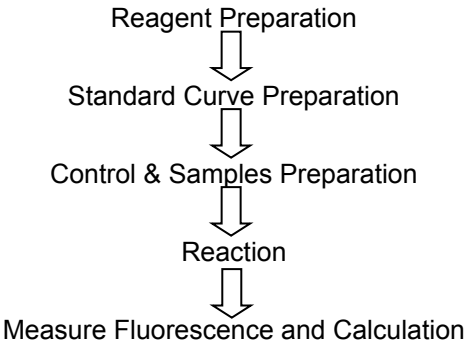
β-Hydroxybutyrate (β-HB) is the most abundant of the ketone bodies (~78% of total ketone bodies in blood). Ketoacidosis (high concentrations of the ketone bodies) can be caused by a variety of conditions, such as diabetes, alcoholism and severe starvation. Ketoacidosis can be fatal if not treated. Additionally, ketogenic diets can increase the concentration of ketone bodies slightly in healthy individuals.

Abcam's β-Hydroxybutyrate Assay Kit (Fluorometric) (ab180876) offers simplicity, enhanced sensitivity, and can be adapted to high-throughput applications. The assay is based on enzymatic oxidation of β-Hydroxybutyrate that results in generation of fluorescent signal (Ex/Em = 535/587 nm) which is directly proportional to the amount of β-Hydroxybutyrate. The kit offers an excellent alternative for measurement of β-Hydroxybutyrate when biological sample quantities are limited or subketogenic levels are suspected. The assay can detect β-Hydroxybutyrate as low as 4 µM in a variety of biological samples.

Figure 1: Assay Procedure



2. Protocol Summary



3. Kits Components

Item	Quantity
Assay Buffer V/β-Hydroxybutyrate Assay Buffer	25 mL
PicoProbe I/PicoProbe	400 µL
β-HB Enzyme Mix/β-Hydroxybutyrate Enzyme Mix (Lyophilized)	1 vial
Developer XI/β-Hydroxybutyrate Substrate Mix (Lyophilized)	1 vial
β-HB Standard/β-Hydroxybutyrate Standard (Lyophilized)	1 vial

4. Storage and Stability

Upon arrival, store the kit at-20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- Distilled water (dH_2O) or MilliQ
- 96-well white plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- 10 kD Spin Columns (ab93349)

6. Reagents Preparation

1. **Assay Buffer V/β-HYDROXYBUTYRATE Assay Buffer:**
   Warm Assay Buffer to room temperature before use.

2. **PicoProbe I/PicoProbe:**
   PicoProbe I/PicoProbe is light sensitive. Warm to room temperature before use. Aliquot & store at -20°C. Stable for two months.

3. **β-HB Enzyme Mix/β-Hydroxybutyrate Enzyme Mix:**
   Reconstitute with 220 µL Assay Buffer V/β-Hydroxybutyrate Assay Buffer. Pipette gently to dissolve. Aliquot & store at -20°C. Keep on ice while in use. Stable for two months.

4. **Developer XI/β-Hydroxybutyrate Substrate Mix:**
   Reconstitute with 220 µL Assay Buffer V/β-Hydroxybutyrate Assay Buffer. Mix well. Aliquot & store at -20°C. Protect from light. Stable for two months.

5. **β-HB Standard/β-Hydroxybutyrate Standard:**
   Reconstitute with 100 µL ddH_2O to generate 10 mM solution. Store at -20°C. Stable for two months.

7. Assay Protocol

1. Sample Preparation

Samples should be deproteinized using a 10 kDa Spin Column (ab93349). Briefly, add sample to the spin column, centrifuge at 10,000 x g for 10 min. at 4°C. Collect the filtrate. Add 2-25 µL of filtrate into desired well(s) in 96-well plate. Adjust the volume to 50 µL/well with Assay Buffer V/β-Hydroxybutyrate Assay Buffer.

NOTE:

- *β-Hydroxybutyrate concentrations can vary over a wide range. In serum, normal range is 0.02 - 0.4 mM that can exceed up to 3 mM in diabetic ketoacidosis & up to 47 mM in alcoholic ketoacidosis. For unknown samples, we recommend to test several doses to ensure the readings are within the Standard Curve range.*
- *For samples having high background, prepare parallel sample well(s) as background control(s). Endogenous compounds may interfere with the assay.*
- *To ensure accurate determination of β-Hydroxybutyrate in the test samples or for samples having low concentration of β-Hydroxybutyrate, we recommend spiking samples with a known amount of β-HB Standard/β-Hydroxybutyrate Standard (400 pmol).*

a) BACKGROUND CONTROL

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

2. Standard Curve Preparation:

a) Dilute the β-HB Standard /β-Hydroxybutyrate Standard to 0.1 mM by adding 10 µL of 10 mM β-HB Standard/β-Hydroxybutyrate Standard to 990 µL of ddH_2O. Mix well.

b) Using the 0.1 mM, prepare a standard curve dilution as follows, in a microplate or microcentrifuge tubes.

β-HB Standard/beta HB standard amount (µl)	Assay Buffer V/beta HB assay buffer (µL)	Total volume prepared	END CONCENTRATION beta HB IN WELL
0	150	150 µl	0 pmol/well
3	147	150 µl	100 pmol/well
6	144	150 µl	200 pmol/well
9	141	150 µl	300 pmol/well
12	138	150 µl	400 pmol/well
15	135	150 µl	500 pmol/well

Add 50 µl of each standard dilution into a well in a 96-well plate to set up standard. Each dilution has enough amount of standard to set up 2 duplicates x 50 µl/well.

3. Reaction Mix:

Prepare enough Reaction Mix for the number of assays to be performed. For each well, prepare 50 µL Mix containing:

	Reaction Mix	Background Control Mix
Assay Buffer V/β-Hydroxybutyrate Assay Buffer	42 µL	44 µL
β-HB Enzyme Mix/β-Hydroxybutyrate Enzyme Mix	2 µL	---
Developer XI/β-Hydroxybutyrate Substrate Mix	2 µL	2 µL
PicoProbe I/PicoProbe	4 µL	4 µL

Add 50 µL of the reaction mix to each well containing the Standards, and test samples. Mix.

* For samples having high background, add 50 µL of Background Control Mix to the sample background control well(s). Mix well.

Mix enough reagents for the number of assays (samples and standards) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency.

We recommend the following calculation:

	Reaction Mix
Assay Buffer V/β-Hydroxybutyrate Assay Buffer	42 µL x (Nb samples + Standards +1)
β-HB Enzyme Mix/β-Hydroxybutyrate Enzyme Mix	2 µL x (Nb samples + Standards +1)
Developer XI/β-Hydroxybutyrate Substrate Mix	2 µL x (Nb samples + Standards +1)
PicoProbe I/PicoProbe	2 µL x (Nb samples + Standards +1)

4. Measurement

a) Incubate the plate at room temperature for 30 min., protected from light.

b) Measure fluorescence (Ex/Em = 535/587 nm) in a microplate reader.

8. Data Analysis

Calculations:

a) Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample reading. Plot the β-HB Standard/β-Hydroxybutyrate Standard Curve. For unspiked samples, apply the corrected OD to the β-HB Standard/β-Hydroxybutyrate Standard Curve to get B nmol of β-Hydroxybutyrate in the sample well.

Sample β-Hydroxybutyrate concentration (C) = B/V X D pmol/µL = nmol/mL or µM

Where:

B is the amount of β-Hydroxybutyrate in the sample well (pmol)
V is the sample volume added into the reaction well (µL)
D is the sample dilution factor

For **spiked samples**, correct for any sample interference by subtracting the sample reading from spiked sample reading.

B-HB amount in sample well (B) =

$$\left(\frac{ODsample(corrected)}{(ODsample + \beta HB\ Std(corrected) - \ (ODsample(corrected))}\right) * \beta\_HB\ Spike(pm$$

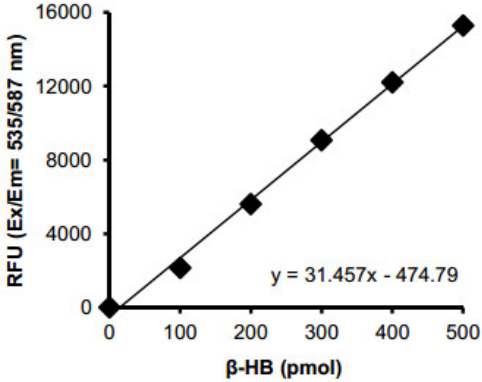


Figure 1. β-Hydroxybutyrate Standard Curve.

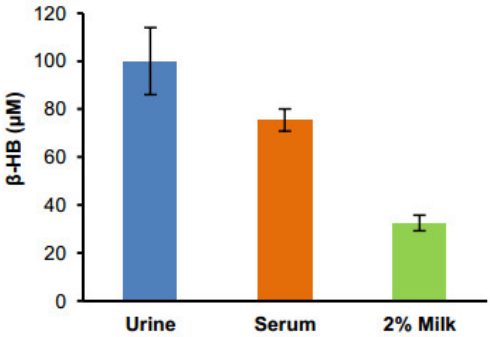


Figure 2. Measurement of β-HB concentration in human urine (2.5 µL), serum (2.5 µL) and 2% Milk (5 µL). All samples were deproteinized using 10 kD Spin Column and spiked with known amount of β-HB (400 pmol).

9. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature

Problem	Reason	Solution
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)