ab83390 beta Hydroxybutyrate A (beta HB) Assay Kit (Colorimetric)

For the rapid, sensitive and accurate measurement of beta- hydroxybutyrate in various samples. This product is for research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab83390
(use abcam.cn/ab83390 for China, or abcam.co.jp/ab83390 for Japan)

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.

Materials Supplied and Storage

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components. Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer V/β-HB Assay Buffer	25 mL	-20°C	-20°C
β-HB Enzyme Mix (lyophilized)	1 vial	-20°C	-20°C
Developer Solution III/β-HB Substrate Mix (ly ophilized)	1 vial	-20°C	-20°C
β-HB Standard (1.0 µmol) (lyophilized)	1 vial	-20°C	-20°C

Materials Required, Not Supplied

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD 450 nm
- 96 well plate: clear plates for colorimetric assay
- Dounce homogenizer or pestle (if using tissue)

For deproteinization step, additional reagents are required:

- Perchloric acid (PCA) 4M, ice cold
- Potassium Hydroxide (KOH) 2M
- 10 kD Spin Columns (ab93349) – for fluid samples, if not performing PCA precipitation

1. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

1.1 **Assay Buffer V/β-HB Assay Buffer:** Ready to use as supplied. Equilibrate to RT before use. Store at -20°C.

1.2 **β-HB Enzyme Mix:** Reconstitute with 220 µL of Assay Buffer. Pipette gently to dissolve. Aliquot enzyme mix so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Once the thawed, use within two months. Keep on ice while in use.

1.3 **Developer Solution III/β-HB Substrate Mix:** Reconstitute with 220 µL of Assay Buffer. Aliquot substrate mix so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Once the thawed, use within two months. Keep on ice while in use.

1.4 **β-HB Standard:** Reconstitute β-HB Standard (1.0 µmol) in 100 µL of ddH_2O to generate a 10 mM standard stock solution. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C.

2. Standard Preparation

Always prepare a fresh set of standards for every use. Diluted standard solution is unstable and must be used within 4 hours.

2.1 Prepare 100 µL of 1mM standard by adding 10 µL of the provided 10 mM standard to 90 µL ddH_2O.

2.2 Using 1mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End [β-HB] in well
1	0	150	50	0 nmol/well
2	6	144	50	2 nmol/well
3	12	138	50	4 nmol/well
4	18	132	50	6 nmol/well
5	24	126	50	8 nmol/well
6	30	120	50	10 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

3. Sample Preparation

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step as well as the deproteinization step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

β-HB concentrations can vary over a wide range from normal range: 20 µM-1 mM to diabetic range: 3-5 mM in serum and 10 times that in urine during diabetic ketoacidosis. Due to the presence of interfering substances in blood and urine up to about 5 µL equivalent of such samples can be tested directly.

3.1 **Cell (adherent or suspension) samples:**

3.1.1 Harvest cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

3.1.2 Wash cells with cold PBS.

3.1.3 Resuspend cells in 100 µL of Assay Buffer V/Assay Buffer.

3.1.4 Homogenize cells quickly by pipetting up and down a few times.

3.1.5 Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

3.1.6 Collect supernatant and transfer to a clean tube.

3.1.7 Keep on ice.

3.1.8 Perform deproteinization step as described in section 3.4.

3.2 **Tissue samples:**

3.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation=10 mg).

3.2.2 Wash tissue in cold PBS.

3.2.3 Resuspend tissue in 100 µL of Assay Buffer V/Assay Buffer.

3.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
3.2.5 Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
3.2.6 Collect supernatant and transfer to a clean tube.
3.2.7 Keep on ice.
3.2.8 Perform deproteinization step as described in section 3.4.

3.3 Plasma, Serum and Urine and other biological fluids:

Due to interfering substances in blood and urine, up to ~5µL of plasma, serum and urine can be tested directly. Add samples to the well and adjust the volume to 50 µL with Assay Buffer V/β-HB Assay Buffer. Alternatively, you can use 10kD Spin column (ab93349) to deproteinize biological fluids. Filtered serum or plasma can be used directly in the assay at 50 µL or up to 100 µL per well. You will not need to adjust the volume with Assay Buffer V/β-HB Assay Buffer in this case. Add samples, then add β-HB Enzyme Mix/enzyme mix and Developer Solution III/substrate mix as described in the assay procedure.

3.4 Deproteinization step:

Prepare samples as specified in protocol. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.

3.4.1 Add ice cold PCA to a final concentration of 1 M in the homogenate solution and vortex briefly to mix well. ***NOTE:*** *high protein concentration samples might need more PCA.*
3.4.2 Incubate on ice for 5 minutes.
3.4.3 Centrifuge samples at 13,000 x g for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube.
3.4.4 Precipitate excess PCA by adding ice-cold 2 M KOH that equals 34% of the supernatant to your samples (for instance, 34 µL of 2 M KOH to 100 µL sample) and vortexing briefly. This will neutralize the sample and precipitate excess PCA. There may be some gas (CO_2) evolution so vent the sample tube.
3.4.5 After neutralization, it is very important that pH equals 6.5 – 8 (use pH paper to test 1 µL of sample). If necessary, adjust the pH with 0.1 M KOH.
3.4.6 Centrifuge at 13,000 x g for 15 minutes at 4°C and collect supernatant.
3.4.7 Transfer supernatant to a clean tube, and keep on ice.

Samples are now deproteinized, neutralized and PCA has been removed. The samples are now ready to use in the assay.

Sample Recovery

The deproteinized samples will be diluted from the original concentration.

To calculate the dilution factor of your final sample, simply apply the following formula:

$$\text{\% original concentration} = \frac{\text{Initial sample volume}}{\text{(initial sample volume + vol PCA + vol KOH)}} \times 100$$

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

4. Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
It is recommended to assay all standards, controls and samples in duplicate.
As reduced pyridine nucleotides NAD(P)H can interfere with the assay, if the presence of these compounds is suspected in the sample, run a background control.

4.1 Set up Reaction wells:

Standard wells = 50 µL standard dilutions.
Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer V/Assay Buffer) depending on sample type and pre-treatment of sample.
Background control sample wells= 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer V/Assay Buffer).

4.2 Reaction Mix:

Prepare 50 µL of Reaction Mix for each reaction

Component	Colorimetric Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer V/β-HB Assay Buffer	46	48
β-HB Enzyme Mix	2	0
Developer Solution III/β-HB Substrate Mix	2	2

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards + background control sample + 1)

4.3 Add 50 µL of appropriate Reaction Mix to each standard, sample and background control sample well.
4.4 Incubate at room temperature for 30 minutes protected from light.
4.5 Measure output on a microplate reader. Colorimetric assay: measure OD450 nm.

5. Calculations:

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

5.1 Average the duplicate reading for each standard and sample.
5.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
5.3 Plot the corrected absorbance values for each standard as a function of the final concentration of β-hydroxybutyrate.
5.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
5.5 Extrapolate sample readings from the standard curve plotted using the following equation:

$$x = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

5.6 Concentration of β-hydroxybutyrate in the test samples is calculated as:

$$X = \left(\frac{A}{B}\right) * D$$

Where:
A = Amount of β-hydroxybutyrate in the sample well (conc).
B = Sample volume added into the reaction well (µL).
D = Sample dilution factor.
β-hydroxybutyrate molecular weight = 104.1 g/mol.

FAQs

Which anticoagulant is recommended for blood collection for this assay? Heparin can be used.

How should plasma be processed for this assay? Plasma sample can be used directly or filtered depending upon the amount of insoluble cloudy components.

I found dehydrogenase was used to oxidize hydroxybutyrate first and then a detection reagent will recognize oxobutyrate. I am wondering whether the detection is specific for oxobutyrate. If there are any ketones, will they be detected also? The detailed assay chemistry is proprietary information. The detection agent does not quantify the oxobutyrate but the byproduct of the reaction by the dehydrogenase. The reaction byproducts are produced proportionally based on the amount of β-hydroxybutyrate in a specific sample. This is why this assay kit is specific for β-hydroxybutyrate and will not recognize other ketones.
Should urine samples be filtered before use? We recommend filtering the urine to remove any particulate matter.

Interferences

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure: Reduced pyridine nucleotides (NAD(P)H).

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