（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K785-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（440-460 nm）

Detection range：0.01-2.00 mmol/L

Elabscience®β-Hydroxybutyrate (Ketone Body) Colorimetric Assay Kit

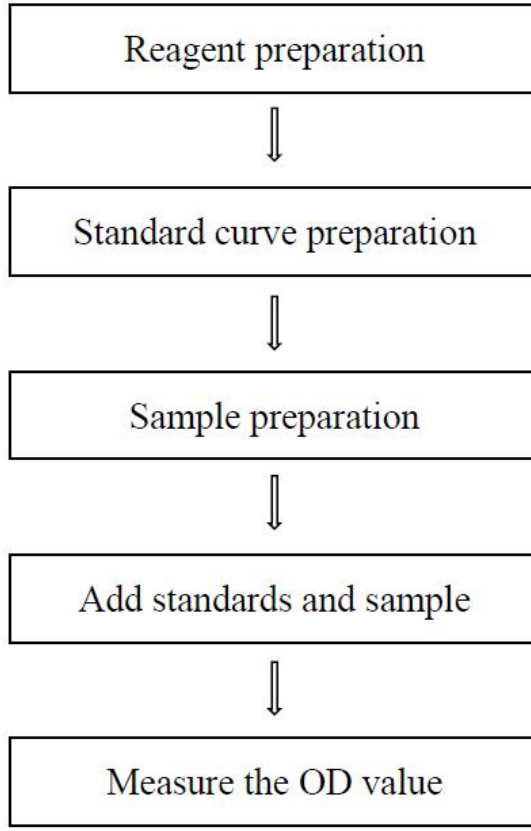
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

This kit can measure β-hydroxybutyrate (β-HB) content in serum, plasma, urine and animal tissue samples.

Detection principle

β-hydroxybutyrate (β-HB), $C_4H_8O_3$, accounts for about 75% of the total ketone body. Patients with diabetic ketoacidosis have increased production of NADH, which promotes the conversion of acetoacetic acid into β-HB. Therefore, the level of β-hydroxybutyrate can be used as an index to evaluate glycosuria. β-hydroxybutyrate dehydrogenase can catalyze the oxidative dehydrogenation of β-HB. Meanwhile, NAD^+ is reduced to NADH, Which under the action of hydrogen transmitter, transfer electrons to WST-8 to produce the yellow product. The content of β-HB can be calculated by measuring the change of absorbance value at 450 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution A	50 mL × 1 vial	50 mL × 2 vials	2-8℃, 12 months, shading light
Reagent 2	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months, shading light
Reagent 3	Buffer Solution B	5 mL × 1 vial	10 mL × 1 vial	2-8℃, 12 months
Reagent 4	Chromogenic Agent	1.5 mL× 1 vial	1.5 mL×2 vials	2-8℃, 12 months, shading light
Reagent 5	10 mmol/L Standard	0.5 mL × 1 vial	1 mL × 1 vial	2-8℃, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain

sufficient amount of reagents.

Materials prepared by users

Instruments:

Incubator, 50 kD Ultrafiltration tube, Microplate reader (440-460 nm, optimum wavelength: 450 nm)

Reagents:

Double distilled water

Reagent preparation

① Keep enzyme reagent on ice during use. Equilibrate other reagents to room temperature before use.

② Preparation of enzyme working solution:
Dissolve one vial of enzyme reagent with 1 mL of double distilled water, mix well to dissolve. Aliquoted store at -20°C for 1 month protected from light.

③ Preparation of enzyme reaction working solution:
For each well, prepare 50 μL of enzyme reaction working solution (mix well 10 μL of enzyme working solution and 40 μL of buffer solution B). Keep enzyme reaction working solution on ice during use. The enzyme reaction working solution should be prepared on spot and used up within the same day.

④ Preparation of chromogenic working solution:
For each well, prepare 160 μL of chromogenic working solution (mix well 140 μL of buffer solution A and 20 μL of chromogenic agent). Keep enzyme reaction working solution on ice during use. The chromogenic working solution should be prepared on spot and used up within the same day.

⑤ Preparation of 5 mmol/L standard solution:
Dilute 150 μL of 10 mmol/L standard with 150 μL of double distilled water, mix well. Store at 2-8°C for 2 days.

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 5 mmol/L standard solution with buffer solution A to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.5, 0.8, 1.0, 1.2, 1.5, 2.0, mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.5**	**0.8**	**1.0**	**1.2**	**1.5**	**2.0**
5 mmol/L standard (μL)	0	8	20	32	40	48	60	80
Buffer Solution A (μL)	200	192	180	168	160	152	140	120

Sample preparation

① Sample preparation

Serum and plasma: Ultrafiltration directly. If the sample is turbidity, centrifuge at 10000×g for 10 min. Centrifuge the supernatant with a 50 kD ultrafiltration tube at 10000×g for 15 min, and preserve the sample on ice for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL double distilled water with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Take the supernatant for centrifugation with a 50 kD ultrafiltration tube at 10000×g for 15 min, and preserve the sample on ice for detection.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Human plasma	1
Rat /Mouse serum	1
Rat /Mouse plasma	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat heart tissue homogenate	1
Human urine	1

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① Buffer Solution A and Chromogenic Agent should be stored with shading light.

② Buffer Solution A and Buffer Solution B cannot be mixed, please operate according to the manual.

Operating steps

① Standard well: Add 10 μL of standard solution with different concentrations to the corresponding wells.

Sample well: Add 10 μL of sample to the corresponding wells.

② Add 50 μL of enzyme reaction working solution to each well.

③ Mix fully with microplate reader for 5 s and incubate at 37℃ for 10 min.

④ Add 160 μL of chromogenic working solution to each well.

⑤ Mix fully with microplate reader for 5 s and incubate at 37℃ for 30 min.

⑥ Measure the OD value of each well at 450 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

$$\beta\text{-HB content (mmol/L)} = (\Delta A - b) \div a \times f$$

2. Tissue sample:

$$\beta\text{-HB content (mmol/kg wet weight)} = (\Delta A - b) \div a \div (m \div V) \times f$$

[Note]

ΔA: $OD_{Sample} - OD_{Blank}$.

m: The weight of the sample, 0.1 g.

V: The volume of homogenate, 0.9 mL.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.55	1.05	1.50
%CV	2.3	2.0	1.7

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.55	1.05	1.50
%CV	3.8	4.2	4.0

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 105%.

	standard 1	standard 2	standard 3
Expected Conc. (mmol/L)	0.25	0.85	1.3
Observed Conc. (mmol/L)	0.3	0.9	1.4
Recovery rate(%)	104	106	105

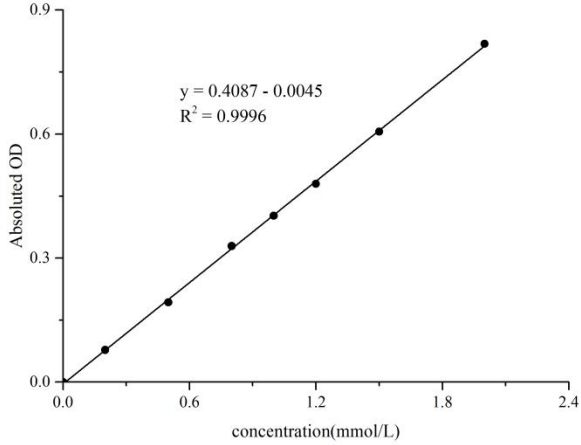
Sensitivity

The analytical sensitivity of the assay is 0.01 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**0.2**	**0.5**	**0.8**	**1.0**	**1.2**	**1.5**	**2.0**
Average OD	0.061	0.139	0.254	0.390	0.463	0.540	0.667	0.879
Absoluted OD	0	0.078	0.193	0.329	0.402	0.479	0.606	0.818



Appendix II Example Analysis

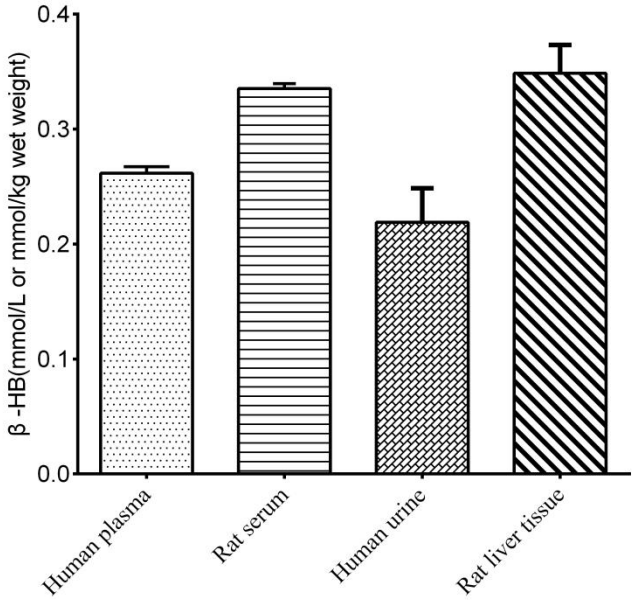
Example analysis：

For rat serum, take 10 μL of rat serum, and carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.5349 x - 0.0025, the average OD value of the blank is 0.094, the average OD value of the sample is 0.319, and the calculation result is:

β-HB content (mmol/L) = (0.319 – 0.094 + 0.0025) ÷ 0.5349 = 0.42 mmol/L

Detect human plasma, rat serum, human urine and 10% rat liver tissue homogenate according to the protocol, the result is as follows：



Appendix III Publications

1. Zhuang H , Ren X , Zhang Y ,et al. β - Hydroxybutyrate enhances chondrocyte mitophagy and reduces cartilage degeneration in osteoarthritis via the HCAR2/AMPK/PINK1/Parkin pathway[J].Aging Cell, 2024, 23(11).DOI:10.1111/acel.14294.
2. He J K , Jiang X X , Dai S Y ,et al. β -Hydroxybutyrate and Citrate Synthase as Potential Diagnostic Biomarkers in Aging-Related Atrial Fibrillation[J].Journal of Cardiovascular Translational Research, 2025, 18(1):133-145.DOI:10.1007/s12265-024-10569-9.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.