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

Catalog No：E-BC-F175

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.57-100 μmol/L

Elabscience® β-Hydroxybutyrate (β-HB)

Fluorometric 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.

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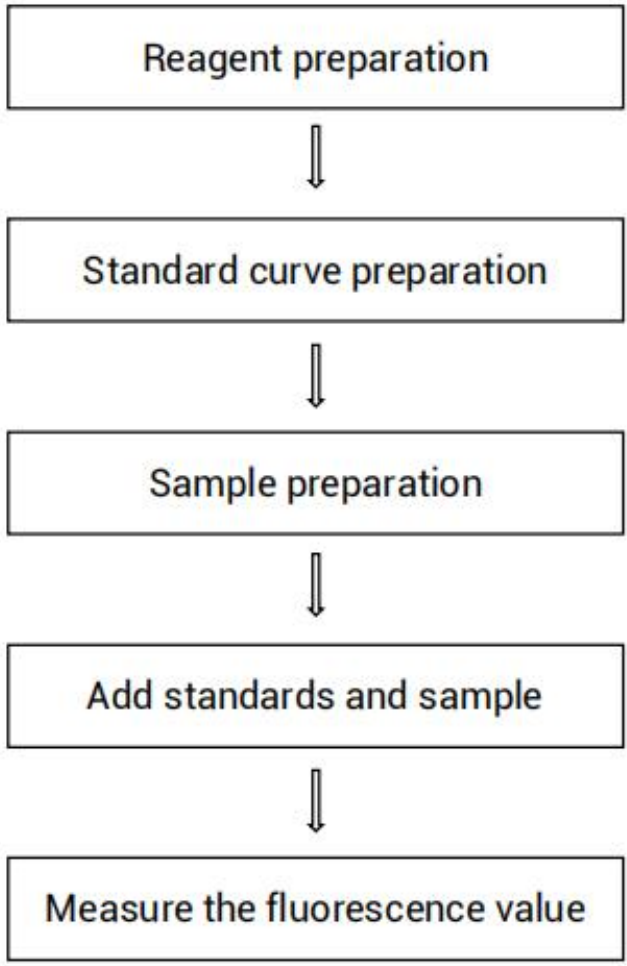
Assay summary



Intended use

The kit is suitable for detecting the content of β-Hydroxybutyrate (β-HB) in serum, plasma, urine, animal tissues and cells.

Detection principle

β-Hydroxybutyrate (β-HB), a short-chain hydroxy fatty acid, together with acetoacetate and acetone, constitutes the three major ketone bodies in the human body. It plays a vital role in human energy metabolism and neuroprotection, and its concentration increases significantly under conditions such as starvation, exercise, and low-carbohydrate diets.

Under the catalysis of β-hydroxybutyrate dehydrogenase, β-HB is oxidized to acetoacetate, during which NAD^+ is reduced to NADH. In the presence of diaphorase, the generated NADH reduces the non-fluorescent resazurin to red-fluorescent resorufin. The production of resorufin in the reaction system is directly proportional to the content of β-HB in the sample. The content of β-HB in the sample can be calculated by measuring the change in fluorescence value.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 2 vials	2-8°C, 12months
Reagent 2	Coenzyme	0.55 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	Chromogenic Agent	0.09 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	Enzyme Reagent A	Power × 2 vials	2-8°C, 12 months, shading light
Reagent 5	Enzyme Reagent B	Power × 1 vial	2-8°C, 12 months, shading light
Reagent 6	10 mM β-Hydroxybutyrate Standard	1 mL × 1 vial	2-8°C, 12 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator,

Materials required but not provided

Distilled or deionized water, 10 kDa MWCO Spin Filter (Outer tube 1.5 mL, Inter tube 0.5 mL)

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② Chromogenic Working Solution preparation：

Before testing, prepare a sufficient Chromogenic Working Solution according to the test wells. For example, prepare 100 μL of Chromogenic Working Solution (mix 99 μL of Buffer Solution and 1 μL of Chromogenic Agent). Stable for 8 h protected from light.

③ Enzyme Reagent A Working Solution preparation：

Dissolve one vial of Enzyme Reagent A with 200 μL of distilled or deionized water. Mix well to dissolve. Stable for one month when stored at -20°C protected from light.

④ Enzyme Reagent B Working Solution preparation：

Dissolve one vial of Enzyme Reagent B with 200 μL of distilled or deionized water. Mix well to dissolve. Stable for one month when stored at -20°C protected from light.

⑤ Working Solution preparation:

Before testing, please prepare sufficient Working Solution. For example, prepare 380 μL of Working Solution (mix well 203 μL of Buffer Solution, 10 μL of Coenzyme, 160 μL of Chromogenic Working Solution, and 7 μL of Enzyme Reagent A Working Solution). The Working Solution should be freshly prepared before use. Stable for 8 h protected from light.

⑥ Control Working Solution preparation：

Before testing, please prepare sufficient Control Working Solution. For example, prepare 380 μL of Working Solution (mix well 203 μL of Buffer Solution, 10 μL of Coenzyme, 160 μL of Chromogenic Working Solution, and 7 μL of Enzyme Reagent B Working Solution). The Working Solution should be freshly prepared before use. Stable for 8 h protected from light.

⑦ 1 mmol/L Standard Solution preparation:

Before testing, prepare a sufficient 1 mmol/L Standard Solution according to the test wells. For example, prepare 1000 μL of 1 mmol/L Standard Solution (mix 900 μL of Buffer Solution and 100 μL of 10 mM β-Hydroxybutyrate Standard thoroughly). Stable for 8 h.

⑧ Standard curve preparation：

Always prepare a fresh set of standards. Discard Working Standard Dilutions after use.

Dilute 1 mmol/L Standard Solution with Buffer Solution to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 30, 40, 60, 80, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**10**	**20**	**30**	**40**	**60**	**80**	**100**
1 mmol/L Standard (μL)	0	10	20	30	40	60	80	100
Buffer Solution (μL)	1000	990	980	970	960	940	920	900

Sample preparation

Serum or plasma samples:

① Add serum or plasma sample into 10kDa MWCO Spin Filter and centrifuge at 10000×g for 30 min.

② Collect the filtrate from the lower filter tube and preserve it on ice for detection and detect within 8 h.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg). Wash tissue in cold PBS (0.01 M, pH 7.4).

② Homogenize 20 mg tissue in 180 μL Buffer Solution with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Transfer the supernatant to 10kDa MWCO Spin Filter. Centrifuge at 10000×g for 30 min. Collect the filtrate from the lower filter tube and preserve it on ice for detection and detect within 8 h.

Cell sample

① Harvest the number of cells needed for each assay (initial recommendation 5×10^6 cells). Wash cells with PBS (0.01 M, pH 7.4).

② Homogenize 5 × 10^6 cells in 200 μL Buffer Solution with a dounce homogenizer.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Transfer the supernatant to 10kDa MWCO Spin Filter. Centrifuge at 10000×g for 30 min. Collect the filtrate from the lower filter tube and preserve it on ice for detection and detect within 8 h.

Dilution of sample

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

Sample type	Dilution factor
Human urine	1
Rat serum	5-10
Human serum	5-10
Mouse serum	10-20
10% Mouse heart tissue homogenization	1
10% Mouse liver tissue homogenization	5-10
10% Mouse kidney tissue homogenization	5-10
5×10^6 HepG2 cells	1

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

Operating steps

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

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

② Add 190 μL of Working Solution into standard well and sample well.

③ Add 190 μL of Control Working Solution into control well.

④ Shake the microplate for 5 seconds to ensure complete mixing. Incubate at 37°C for 0 min. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using corrected fluorescence 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 or plasma samples:

$$\text{β-HB content (μmol/L)} = (\Delta F - b) \div a \times f$$

2. Tissue sample:

$$\text{β-HB content (μmol/kg wet weight)} = (\Delta F - b) \div a \times V \div m \times f$$

3. Cell sample:

$$\text{β-HB content (nmol/10^9)} = (\Delta F - b) \div a \times V \div n \times f \times 1000$$

[Note]

ΔF: $\Delta F = F_{sample} - F_{control}$

m: The weight of tissue, kg.

V: The volume of Buffer solution added to sample homogenization, L.

n: The number of cell sample/10^9.

f: Dilution factor of sample before tested.

1000: 1 μmol/L=1000 nmol/L.

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 (μmol/L)	16	49	88
%CV	1.4	1.4	1.5

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 (μmol/L)	16	49	88
%CV	2.3	3.8	3.1

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 103.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	16	49	88
Observed Conc. (μmol/L)	16.16	49.49	89.76
Recovery rate (%)	101	101	102

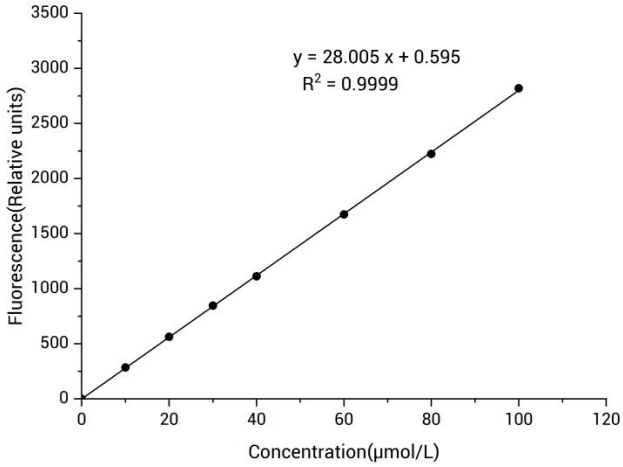
Sensitivity

The analytical sensitivity of the assay is 0.57 μmol/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 fluorescence 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 (μmol/L)	**0**	**10**	**20**	**30**	**40**	**60**	**80**	**100**
Fluorescene value	570	856	1122	1411	1666	2245	2797	3395
	565	851	1140	1418	1695	2238	2786	3378
Average fluorescene value	568	854	1131	1415	1681	2242	2792	3387
Corrected fluorescene value	0	286	564	847	1113	1674	2224	2819



Appendix Π Example Analysis

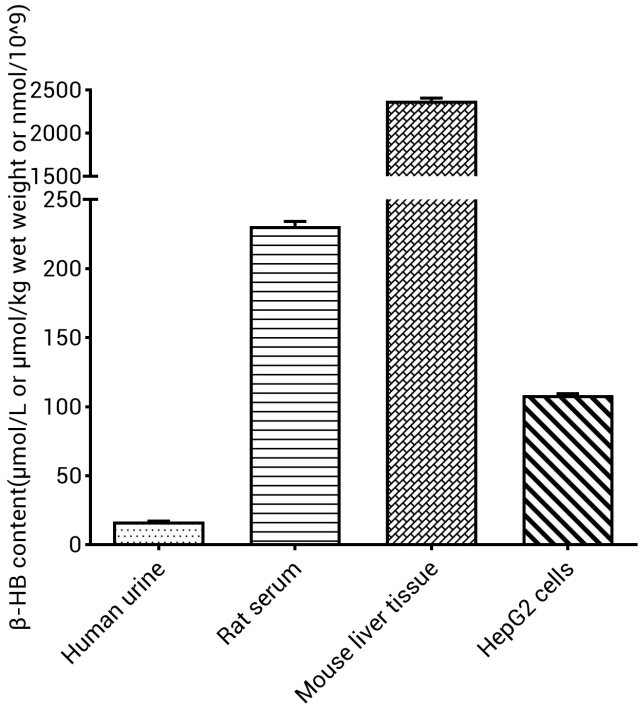
Example analysis：

Take 10 μL of human urine filtrate to the well of microplate. Proceed according to the operating steps. The results are as follows:

standard curve: y = 28.005 x + 0.595, the average fluorescence value of the sample well is 1151, the average fluorescence value of the control well is 672, $\Delta F = F_{sample} - F_{control}$ = 1151 - 672 = 479, and the calculation result is:

β-HB (μmol/L)=(479 - 0.595)÷ 28.005 = 17.08 μmol/L

Detect human urine, rat serun (dilute for 10 times), 10% mouse liver tissue homogenate supernatant (dilute for 10 times), and 5×10^6 HepG2 cells according to the protocol, the result is as follows：



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.