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

Catalog No：E-BC-K1102-M

Specification：48T (46 samples)/96T (94 samples)

Measuring instrument：Microplate reader (340 nm)

Detection range：5.97-1313.73 U/L

Elabscience®α-Hydroxybutyrate Dehydrogenase (α-HBDH) Activity 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.

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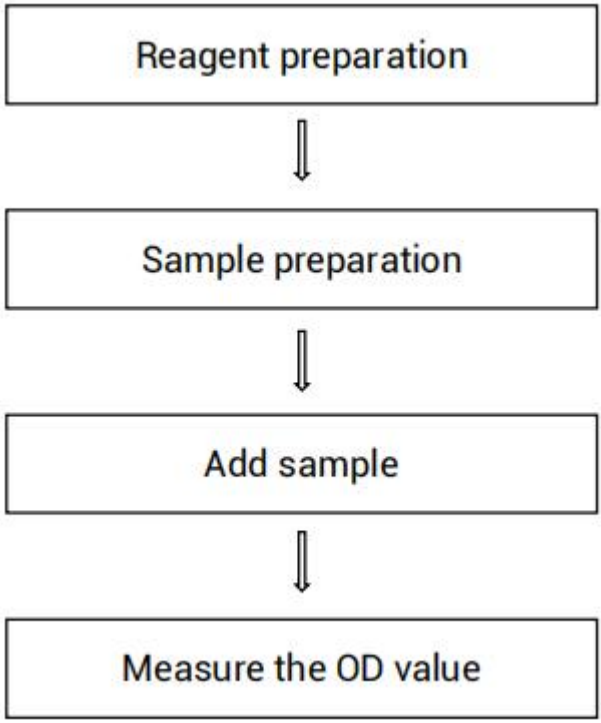
Assay summary



Intended use

This kit can be used to measure α-Hydroxybutyrate dehydrogenase (α-HBDH) activity in serum and plasma samples.

Detection principle

α-Hydroxybutyrate Dehydrogenase (α-HBDH) is a general term for Lactic Dehydrogenase (LDH) isoenzymes LDH 1 and LDH 2, and is one of the five components of the myocardial enzyme spectrum. The increase of serum creatinine is common in myocardial infarction, liver parenchymal cell lesions, rheumatic myocarditis, viral myocarditis, hemolytic anemia and other diseases.

α-HBDH catalyzes the reduction of α-ketobutyric acid to α-hydroxybutyric acid in the presence of reduced coenzyme Ⅰ (NADH), and NADH is oxidized to NAD+ at a rate proportional to the enzyme activity. The activity of α-HBDH can be calculated by monitoring the rate of NADH reduction at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	12 mL × 1 vial	24 mL × 1 vial	2-8°C, 12 months,
Reagent 2	Coenzyme	3 mL × 1 vial	6 mL × 1 vial	2-8°C, 12 months
	UV-Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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:

Microplate reader (340 nm), Incubator

Reagents:

PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of reaction working solution:

Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 250 μL of reaction working solution (mix well 200 μL of s buffer solution and 50 μL of coenzyme). The reaction working solution should be prepared on spot, and the prepared solution should be used up on the same day.

Sample preparation

① Sample preparation

Serum or plasma samples: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

② Dilution of sample

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

Sample type	Dilution factor
Human serum	1
Mouse plasma	1
Human plasma	1
Rat plasma	1

Note: The diluent is PBS (0.01 M，pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Blank well: Add 10 μL of double distilled water to the corresponding well.

Sample well: Add 10 μL of samples to the corresponding well.

② Add 250 μL of reaction working solution to each well.

③ Mix fully with microplate reader for 5 s and incubate at 37℃ for 3 min. Measure the OD value of each well at 340 nm with microplate reader, as A_1.

Incubate at 37°C for 5 min, measure the OD value of each well at 340 nm with microplate reader, as A_2, $\Delta A = A_1 - A_2$.

Calculation

The sample:

Serum and plasma samples:

Definition: The amount of enzyme in 1 L serum (plasma) per 1 min that produce 1 μmol production at 37°C is defined as 1 unit.

$$\text{α-HBDH activity (U/L)} = (\Delta A_{sample} - \Delta A_{blank}) \div (\varepsilon \times d) \times \frac{V_{total}}{V_{sample}} \div T \times f$$

$$= (\Delta A_{sample} - \Delta A_{blank}) \times 1194.3* \times f$$

[Note]

ΔA_{sample}: Absolute OD value of the sample, $A_1 - A_2$.

ΔA_{blank}: Absolute OD value of the blankl, $A_1 - A_2$.

ε: The molar extinction coefficient, 6.22×10^(-3) L·μmol-1·cm-1

d: Optical path of microplate wells, 0.7 cm.

V_{total}： The volume of reaction system, 260 μL.

V_{sample}： The volume of sample, 10 μL.

T: Reaction time, 5 min.

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 (U/L)	57.1	250.8	481.5
%CV	3.6	2.3	2.6

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 (U/L)	73.5	149.4	287.7
%CV	7.4	2.4	4.0

Sensitivity

The analytical sensitivity of the assay is 5.97 U/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.

Appendix Π Example Analysis

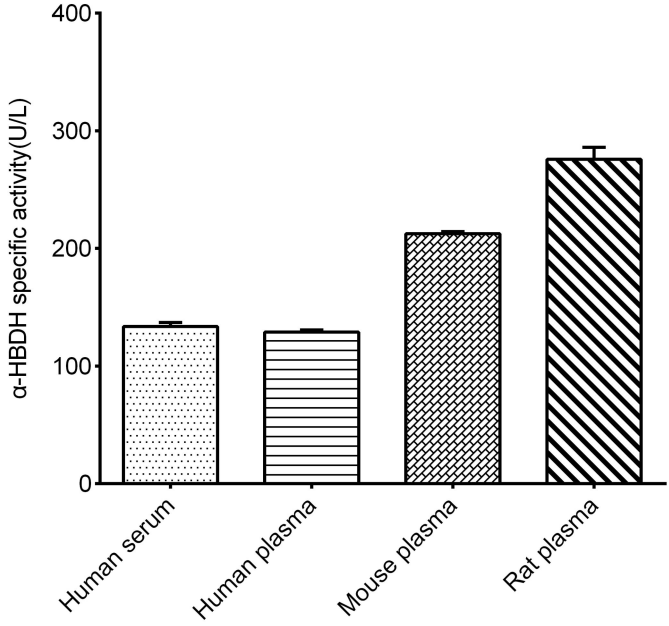
Example analysis：

Take 10 μL of human serum, and carry the assay according to the operation steps. The results are as follows:

The A_1 value of the blank is 1.405, the A_2 value of the blank is 1.403, ΔA_{blank} = 1.405 - 1.403 = 0.002; the A_1 value of the sample is 1.314, the A_2 value of the sample is 1.200, ΔA_{sample} = 1.314 - 1.200 = 0.114, and the calculation result is:

α-HBDH activity(U/L) = (0.114 - 0.002) × 1194.3 = 133.76 U/L

Detect human serum, human plasma, mouse plasma, rat plasma, 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.