TECHNICAL MANUAL

alpha-Hydroxybutyrate Dehydrogenase (alpha-HBDH) Activity Colorimetric Assay Kit

- **SKU CODE:** MAES0376
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

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

2. Detection principle

α-Hydroxybutyrate Dehydrogenase (α-HBDH) is a general term for Lactic Dehydrogenase (LDH) isoenzymes LDH1 and LDH2, and is one of the five components of the myocardial enzyme spectrum. Elevated serum α-HBDH levels are commonly observed 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.

3. Kit components & storage

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

4. Materials prepared by users

Instruments:

Microplate reader (340 nm), Incubator

Reagents:

PBS (0.01 M, pH 7.4)

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 small volume reagents, please centrifuge before use to ensure sufficient reagent recovery.

5. Reagent preparation

1. Equilibrate all reagents to 25 °C before use.
2. **Preparation of reaction working solution:** Before testing, prepare sufficient reaction working solution according to the number of test wells. For example, to prepare 250 µL of reaction working solution, mix 200 µL of buffer solution with 50 µL of coenzyme and mix well. The reaction working solution should be prepared fresh and used on the same day.

6. Sample preparation

1. **Sample preparation:** Serum or plasma samples: detect directly. If not detected on the same day, serum or plasma can be stored at -80 °C for one 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 dilution of other sample types, please perform a pretest to confirm the dilution factor.

7. Operating steps

1. Blank well: Add 10 µL of double distilled water to the corresponding well. Sample well: Add 10 µL of sample to the corresponding well.
2. Add 250 µL of reaction working solution to each well.
3. Mix thoroughly with microplate reader for 5 seconds and incubate at 37 °C for 3 minutes. Measure the OD value of each well at 340 nm with microplate reader, record as A1.
4. Incubate at 37 °C for 5 minutes, then measure the OD value of each well at 340 nm with microplate reader, record as A2. Calculate ΔA = A1 - A2.

8. Calculation

The sample:

Serum and plasma samples:

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

α-HBDH activity (U/L) = (ΔA_sample - ΔA_blank) ÷ (ε × d × V_{total}/V_{sample}) ÷ T × f

= (ΔA_sample - ΔA_blank) × 1194.3* × f

[Note]

ΔA_sample: Absolute OD value of the sample, A_1 - A_2.

ΔA_blank: Absolute OD value of the blank, 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.

9. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

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.

10. Appendix II Example Analysis

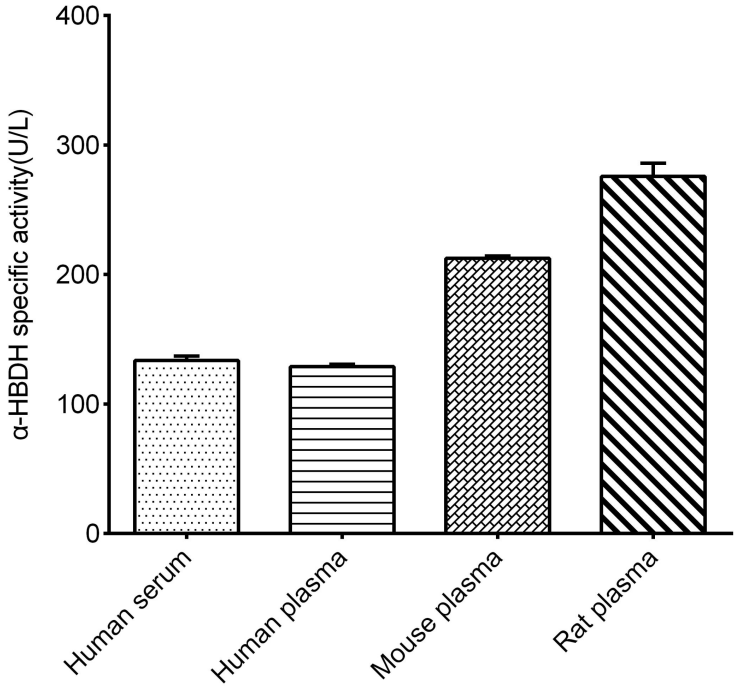
Example analysis:

Take 10 µL of human serum and perform the assay according to the operating 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:



11. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pretest if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.