TECHNICAL MANUAL

β-Hydroxybutyrate (Ketone Body) Colorimetric Assay Kit

- **SKU CODE:** MAES0217
- **SIZE:** 48T / 96T
- **PRODUCT TYPE:** Colorimetric
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Key Features

Detection Range: 0.01-2.00 mmol/L

Measuring Instrument: Microplate reader(440-460 nm)

2. Intended use

This kit enables quantitative measurement of β-hydroxybutyrate (β-HB) content in serum, plasma, urine, and animal tissue samples.

3. Detection principle

β-hydroxybutyrate (β-HB), C4H8O3, accounts for approximately 75% of total ketone bodies. Patients with diabetic ketoacidosis exhibit increased NADH production, which promotes the conversion of acetoacetic acid to β-HB. Therefore, β-hydroxybutyrate levels serve as an important index for evaluating ketosis. β-hydroxybutyrate dehydrogenase catalyzes the oxidative dehydrogenation of β-HB, simultaneously reducing NAD+ to NADH. Through the action of an electron carrier, NADH transfers electrons to WST-8, producing a yellow product. The β-HB content is determined by measuring the absorbance change at 450 nm.

4. Kit components & storage

Store all components under the conditions specified in the table above. Reagents from different kits must not be mixed. For small volume reagents, centrifuge briefly before use to ensure adequate reagent recovery.

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

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Microplate	48 wells	96 wells	No requirement

5. Materials prepared by users

Instruments

Incubator, 50 kD ultrafiltration tubes, microplate reader (440-460 nm, optimal wavelength: 450 nm)

Reagents

Double distilled water

6. 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 and mix well until completely dissolved. Store aliquots at -20°C for up to 1 month, protected from light.

3. Preparation of enzyme reaction working solution:

For each well, prepare 50 µL of enzyme reaction working solution by mixing 10 µL of enzyme working solution with 40 µL of buffer solution B. Keep enzyme reaction working solution on ice during use. Prepare fresh on the day of use.

4. Preparation of chromogenic working solution:

For each well, prepare 160 µL of chromogenic working solution by mixing 140 µL of buffer solution A with 20 µL of chromogenic agent. Keep chromogenic working solution on ice during use. Prepare fresh on the day of use.

5. Preparation of 5 mmol/L standard solution:

Dilute 150 µL of 10 mmol/L standard with 150 µL of double distilled water and mix well. Store at 2-8°C for up to 2 days.

6. Preparation of standard curve:

Always prepare fresh standards. Discard working standard dilutions after use.

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

7. Sample preparation

Serum and plasma

Process by ultrafiltration directly. If the sample is turbid, centrifuge at 10,000×g for 10 min. Centrifuge the supernatant using a 50 kD ultrafiltration tube at 10,000×g for 15 min, and keep the filtrate on ice for detection.

Tissue sample

1. Harvest the required amount of tissue 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 using a Dounce homogenizer at 4°C.
4. Centrifuge at 10,000×g for 10 minutes to remove insoluble material. Take the supernatant and centrifuge using a 50 kD ultrafiltration tube at 10,000×g for 15 min. Keep the filtrate on ice for detection.

Dilution of sample

The recommended dilution factors for different samples are as follows (for reference only):

For other sample types, perform a pretest to determine the appropriate dilution factor.

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

8. The key points of the assay

1. Buffer Solution A and Chromogenic Agent must be stored protected from light.
2. Buffer Solution A and Buffer Solution B must not be mixed. Follow the manual instructions precisely.

9. Operating steps

1. Standard wells: Add 10 µL of standard solution at different concentrations to the corresponding wells. Sample wells: Add 10 µL of sample to the corresponding wells.
2. Add 50 µL of enzyme reaction working solution to each well.
3. Mix thoroughly using the microplate reader for 5 seconds and incubate at 37°C for 10 min.
4. Add 160 µL of chromogenic working solution to each well.
5. Mix thoroughly using the microplate reader for 5 seconds and incubate at 37°C for 30 min.
6. Measure the OD value of each well at 450 nm using a microplate reader.

10. Calculation

The standard curve

1. Average the duplicate readings for each standard.
2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This gives the absolute OD value.
3. Plot the standard curve using the absolute OD values of standards as the y-axis and corresponding concentrations as the x-axis. Generate the

y = ax + b

standard curve (y = ax + b) using graphing software or Excel.

The sample

Serum (plasma) sample:

β-HB content = (ΔA - b) ÷ a × f (mmol/L)

2. Tissue sample:

β-HB content (mmol/kg wet weight) = (ΔA - b) ÷ a ÷ (m ÷ V) × f

[Note]

ΔA: ODSample – ODBlank.

m: Weight of the sample, 0.1 g.

V: Volume of homogenate, 0.9 mL.

f: Dilution factor of sample before testing.

11. Appendix I Performance Characteristics

Intra-assay Precision

Three human serum samples were assayed in 20 replicates 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

Three samples of high, medium, and low concentrations were tested with 6 replicates each to determine the average recovery rate of 105%.

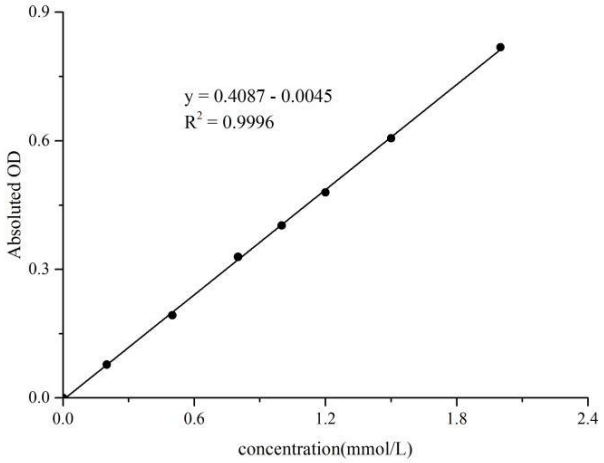
standard 1	standard 2	standard 3
0.25	0.85	1.3
0.3	0.9	1.4
104	106	105

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.

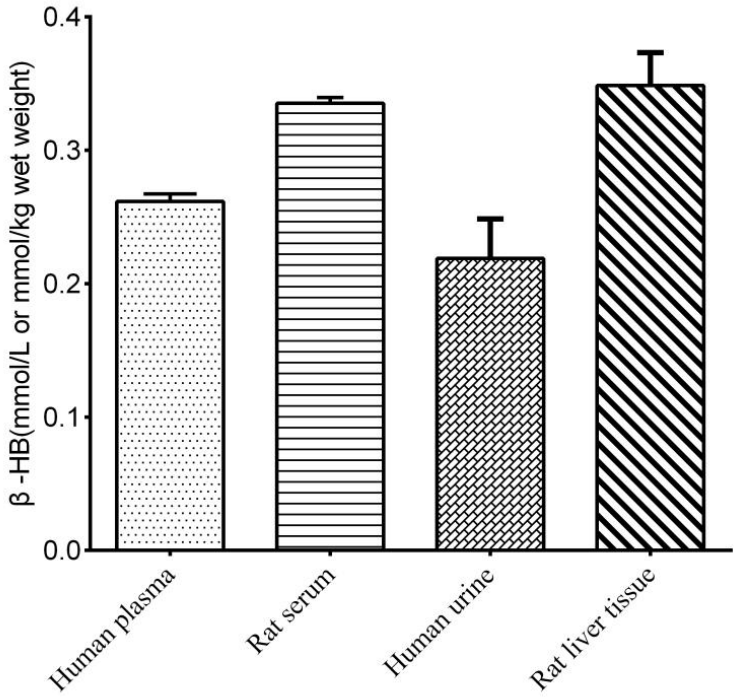
2. Standard curve

The OD values of the standard curve may vary according to actual assay conditions (e.g., operator, pipetting technique, or temperature effects). The standard curve and data provided below are 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

12. Appendix II Example Analysis



Example analysis

For rat serum, take 10 μL of rat serum and perform the assay according to the operating procedure. The results are as follows:

Standard curve: y = 0.5349x - 0.0025, average OD value of blank is 0.094, average OD value of 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

Detection of human plasma, rat serum, human urine, and 10% rat liver tissue homogenate according to the protocol yielded the following results:

13. Statement

Assay Genie shall not be held responsible for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or other purposes.

2. Please read the instructions carefully and calibrate instruments before experiments. Follow the instructions strictly during experiments.

3. Protective measures must be taken by wearing laboratory coats and latex gloves.

4. If the analyte concentration is not within the detection range, additional dilution or concentration of the sample should be performed.

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 conditions, operations, environment, and other factors. Assay Genie guarantees only the quality of the kits and is NOT responsible for sample consumption caused by using the assay kits. It is advisable to calculate the possible sample usage and reserve sufficient samples before use.

Notes:

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.