PRODUCT INFORMATION & MANUAL

beta-Hydroxybutyrate Assay Kit (Colorimetric)

NBP3-25919

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

beta-Hydroxybutyrate Assay Kit (Colorimetric)

Catalog No: NBP3-25919

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.01 mmol/L

Detection range: 0.01-2.00 mmol/L

Average intra-assay CV (%): 2

Average inter-assay CV (%): 4

Average recovery rate (%: 105

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲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	Specification	Storage
Reagent 1	Buffer Solution A	50 mL × 2 vials	2-8℃ , 12 months, shading light
Reagent 2	Enzyme Reagent	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 3	Buffer Solution B	10 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Chromogenic Agent	1.5 mL × 2 vials	2-8℃ , 12 months, shading light
Reagent 5	10 mmol/L Standard	1 mL × 1 vial	2-8℃ , 12 months
	Microplate	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.			

▲Materials prepared by users

Instruments

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

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. Reagent 1 and reagent 4 should be stored with shading light.
2. Reagent 1 and reagent 3 cannot be mixed, please operate according to the manual.

Pre-assay preparation

▲ Reagent preparation

1. Preserve reagent 2 on ice for detection, and bring other reagents to room temperature before use.
2. Preparation of reagent 2 working solution:
   Dissolve a vial of reagent 2 with 1 mL of double distilled water and mix fully, preserve it on ice with shading light for detection. The prepared solution can be stored at -20℃ for one month with shading light.
3. Preparation of reagent 2 reaction working solution:
   Mix the reagent 2 working solution and reagent 3 at the ratio of 1:4 fully and preserve it on ice with shading light for detection. Prepare the fresh needed amount before use and the prepared solution should be used on the same day.
4. Preparation of reagent 4 working solution:
   Mix the reagent 1 and reagent 4 at the ratio of 7:1 fully and preserve it on ice with shading light for detection. Prepare the fresh needed amount before use and the prepared solution should be used on the same day.
5. Preparation of 5 mmol/L standard solution:
   Dilute the reagent 5 with double distilled water at a ratio of 1:1. Prepare the fresh needed amount before use. The prepared solution can be stored at 2-8℃ for 2 days.

▲ Sample preparation

1. Tissue sample:
   Accurately weigh the tissue, add double distilled water at a ratio of weight (g): volume (mL) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 15 min, 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. Serum, plasma and urine samples:

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.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.01-2.00 mmol/L)).

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.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operating steps

1. The preparation of standard curve

Dilute 5 mmol/L standard solution with reagent 1 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:

Number	Standard concentrations (mmol/L)	5 mmol/L Standard (μL)	Reagent 1 (μL)
A	0	0	200
B	0.2	8	192
C	0.5	20	180
D	0.8	32	168
E	1.0	40	160
F	1.2	48	152
G	1.5	60	140
H	2.0	80	120

2. The measurement of samples

(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 reagent 2 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 reagent 4 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.

▲Summary operation table

	Standard well	Sample well
Standard solution with different concentrations (μL)	10	
Sample (μL)		10
Reagent 2 reaction working solution (μL)	50	50
Mix fully and incubate at 37℃ for 10 min.		
Reagent 4 working solution (μL)	160	160
Mix fully and incubate at 37℃ for 30 min. Measure the OD value at 450 nm.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Tissue :

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

2. Serum/plasma sample:

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

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of Standard.

a: The slope of standard curve.

b: The intercept of standard curve.

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 tested.

Appendix I Data

▲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