beta-Hydroxybutyrate

Microplate Assay Kit

User Manual

Catalog # CAK1309

(Version 1.1A)

Detection and Quantification of beta-Hydroxybutyrate (β-HB) Content in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

Diabetic ketoacidosis occurs when circulating insulin levels drop to very low levels, shutting off the supply of glucose to the body. The physiological response is for the liver to produce ketone bodies (acetoacetate, acetone, and primarily β-hydroxybutyrate) from the acetyl CoA produced from fatty acid oxidation. The very high rate of ketone body production outstrips the body's ability to utilize them as an energy source and the blood concentration builds up. As rather strong acids, they result in a significant drop in blood pH.

beta-Hydroxybutyrate Microplate Assay Kit provides a simple and direct procedure for measuring beta-Hydroxybutyrate concentration in a variety of samples. This assay kit utilizes beta-Hydroxybutyrate Dehydrogenase to generate a product which reacts with our colorimetric probe with an absorbance band at 450 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Reaction Buffer to dissolve before use, store at -80 °C.

Dye Reagent A: add 9 ml distilled water to dissolve before use, mix, store at 4°C.

Standard: add 1 ml distilled water to dissolve before use; then add 200 µl into 800 µl distilled water, the concentration will be 5 mmol/L, store at -20 °C.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	80 µl	80 µl	80 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Enzyme	10 µl	10 µl	10 µl
Mix.			
Dye Reagent A	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl
Mix, incubate at room temperature for 5 minutes, record absorbance measured at 450 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

$$\beta\text{-HB}\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

$$\beta\text{-HB}\ (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$\beta\text{-HB}\ (\mu mol/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$$

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the concentration of standard, 5 mmol/L = 5 μmol/ml;

$V_{Standard}$: the volume of standard, 10 μl = 0.01 ml;

V_{Sample}: the volume of sample, 10 μl = 0.01 ml;

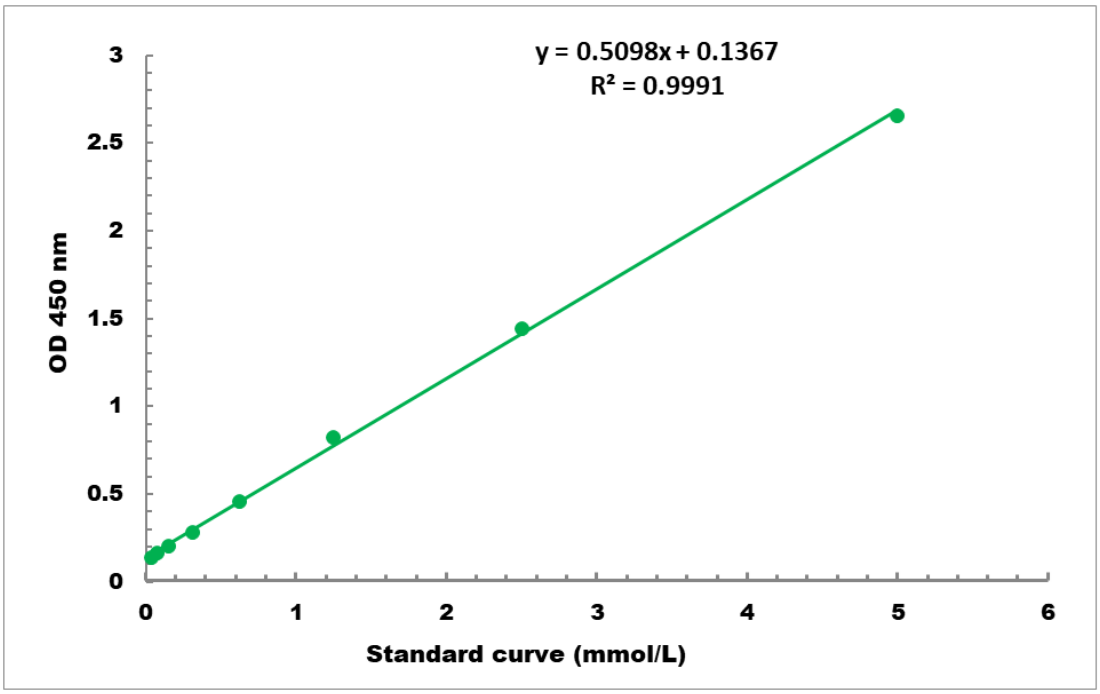
V_{Assay}: the volume of Assay buffer, 1 ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.05 mmol/L - 5 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES