



beta-Hydroxybutyrate Dehydrogenase

Microplate Assay Kit

User Manual

Catalog # CAK1310

(Version 1.1A)

Detection and Quantification of beta-Hydroxybutyrate

Dehydrogenase (β -HBDH) Activity 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.

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I. INTRODUCTION

beta-Hydroxybutyrate Dehydrogenase (EC 1.1.1.30), also known as BHBDH, is found in the mitochondria and catalyzes the oxidation of 3-hydroxybutyrate to acetoacetate and it uses NAD as a coenzyme. This enzyme catalyzes the reaction of (R)-3-hydroxybutanoate and NAD⁺ into acetoacetate into NADH and a free H⁺.

beta-Hydroxybutyrate Dehydrogenase Microplate Assay Kit provides a simple and direct procedure for measuring beta-Hydroxybutyrate Dehydrogenase activity in a variety of samples. In this colorimetric beta-Hydroxybutyrate Dehydrogenase quantification assay, beta-Hydroxybutyrate Dehydrogenase reduces NAD to NADH, which then interacts with a specific probe to produce a color. The rate of increase in the absorbency at 450 nm, is a measure of beta-Hydroxybutyrate Dehydrogenase activity.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	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
Positive Control	Powder x 1	-20 °C
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Note:

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

Substrate: add 9 ml Assay Buffer to dissolve before use, store at -20 °C.

Standard: add 1 ml distilled water to dissolve before use; then add 0.15 ml into 0.85 ml distilled water, the concentration will be 300 µmol/L, store at -20 °C.

Positive Control: add 1 ml Assay Buffer to dissolve before use, then add 0.1 ml into 0.9 ml Assay Buffer, store at -80 °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	Control	Standard	Blank	Positive Control
Substrate	90 μ l	90 μ l	--	--	90 μ l
Sample	10 μ l	--	--	--	--
Standard	--	--	100 μ l	--	--
Positive Control	--	--	--	--	10 μ l
Distilled water	--	10 μ l	--	100 μ l	--
Mix.					
Dye Reagent A	90 μ l	90 μ l	90 μ l	90 μ l	90 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l
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) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- 3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of β -HBDH activity is defined as the enzyme produce 1 μmol NADH per minute.

1. According to the volume of serum or plasma

$$\begin{aligned}\beta\text{-HBDH (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &V_{\text{Sample}} / T \\ &= 0.6 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}})\end{aligned}$$

2. According to the protein concentration of sample

$$\begin{aligned}\beta\text{-HBDH (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &(V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 0.6 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / C_{\text{Protein}}\end{aligned}$$

3. According to the weight of sample

$$\begin{aligned}\beta\text{-HBDH (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / (W \times \\ &V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 0.6 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / W\end{aligned}$$

4. According to the quantity of cell or bacteria

$$\begin{aligned}\beta\text{-HBDH (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / (N \\ &\times V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 0.6 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / N\end{aligned}$$

C_{Standard} : the concentration of standard, $300 \mu\text{mol/L} = 0.3 \mu\text{mol/ml}$;

C_{Protein} : the protein concentration, mg/ml ;

W: the weight of sample, g;

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

V_{Standard} : the volume of standard, 0.1 ml;

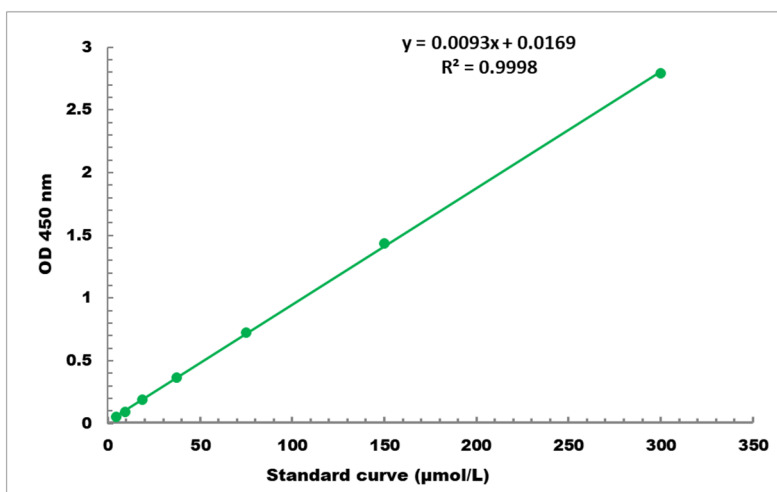
V_{Assay} : the volume of Assay buffer, 1 ml;

T: the reaction time, 5 minutes;

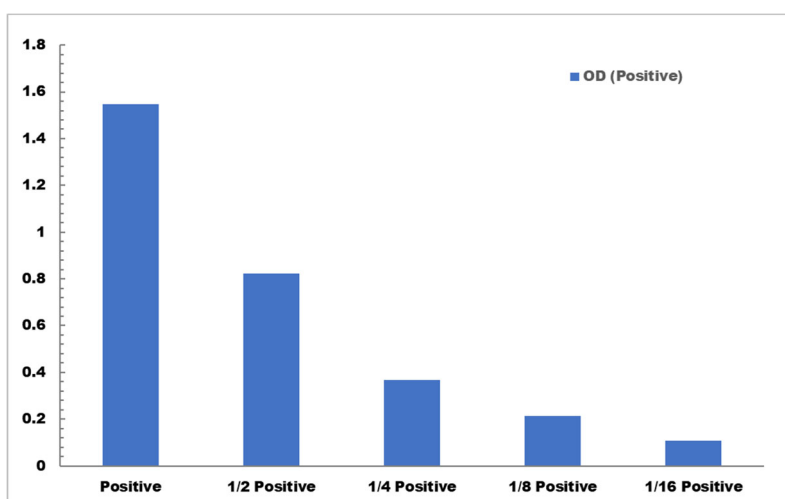
N: the quantity of cell or bacteria, $N \times 10^4$.

VII. TYPICAL DATA

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



Detection Range: 3 μmol/L - 300 μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

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

IX. NOTES