



beta-Hydroxybutyrate Dehydrogenase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1310

(Version 1.2A)

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 Activity Colorimetric 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 ReagentA	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
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 20 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 0.3 mmol/L standard top solution by adding 0.15 ml stock solution into 0.85 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 300/150/75/37.5/18.8/9.4/4.7 $\mu\text{mol/L}$.

Dye Reagent A: Add 9 ml distilled water to dissolve before use, mix. Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

Substrate: Add 9 ml Assay Buffer to dissolve before use. Keep in dark and store at 4°C for 1 week or -20 °C for 2 months.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use, then add 0.1 ml into 0.9 ml Assay Buffer. Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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.

VI. 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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of β -HBDH activity is defined as the enzyme produce $1\mu\text{molNADH}$ per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n(\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein} : the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

C_{Standard} : the concentration of standard, $\mu\text{mol/mL}$

V_{Standard} : the volume of standard in assay procedure, mL

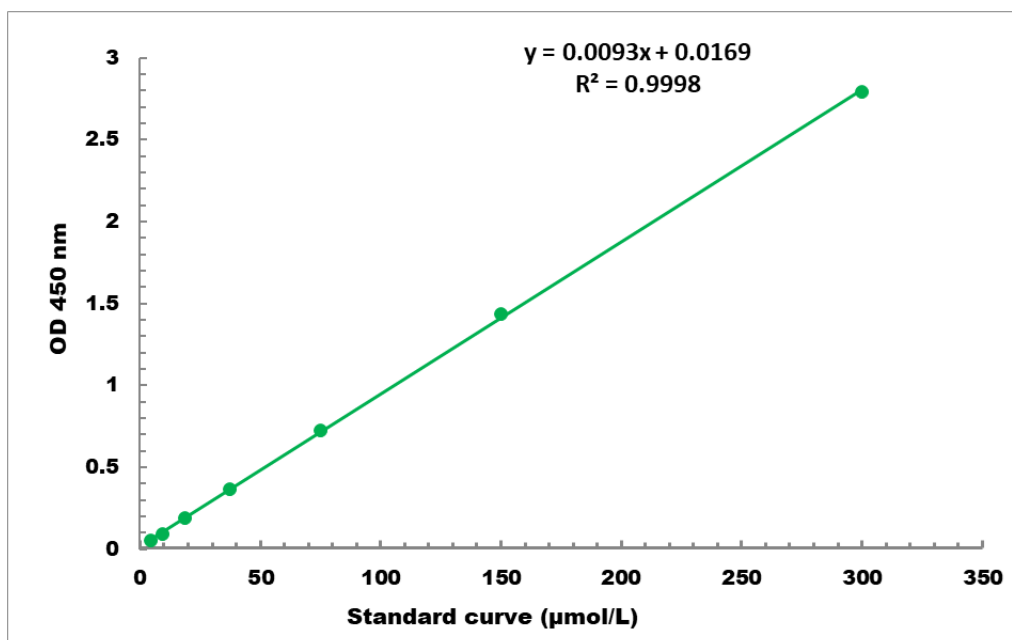
V_{Sample} : the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

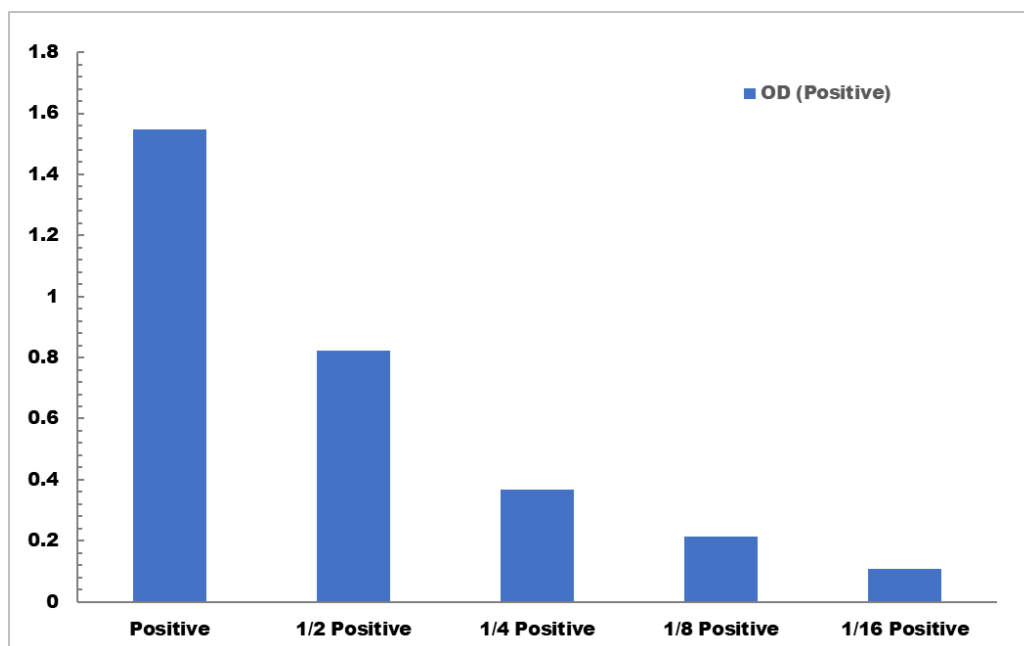
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 3µmol/L -300 µmol/L



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