# alpha-Hydroxybutyrate Dehydrogenase Microplate Assay Kit

# User Manual

## Catalog # CAK1303

(Version 1.1A)

Detection and Quantification of alpha-Hydroxybutyrate Dehydrogenase (α-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.**

## I. INTRODUCTION

α-hydroxybutyrate dehydrogenase (α-HBDH) is an auxiliary marker of myocardial injury, which was reported to have an increased specificity for detecting myocardial injury, starting to increase 8-12 h after damage, reaching peak serum concentrations after 48-72 h and returning to baseline after 7-14 days.

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

## 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 |
| Substrate I | Powder x 1 | -20 °C |
| Substrate II | 1 ml x 1 | 4 °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 |
| Technical Manual | 1 Manual | |

**Note:**

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

**Substrate I**: add 1 ml Reaction 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 0.1 ml distilled water to dissolve before use, 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 $5 \times 10^6$ 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 |
|---|---|---|---|---|---|
| Sample | 10 µl | -- | -- | -- | -- |
| Standard | -- | -- | 100 µl | -- | -- |
| Positive Control | -- | -- | -- | -- | 10 µl |
| Reaction Buffer | 70 µl | 70 µl | -- | -- | 70 µl |
| Substrate I | 10 µl | 10 µl | -- | -- | 10 µl |
| Substrate II | 10 µl | 10 µl | -- | -- | 10 µl |
| Distilled water | -- | 10 µl | -- | 100 µl | -- |
| Mix. | | | | | |
| Dye Reagent A | 90 µl | 90 µl | 90 µl | 90 µl | 90 µl |
| Dye Reagent B | 10 µl | 10 µl | 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) 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 α-HBDH activity is defined as the enzyme reduce 1 μmol NADH per minute.

1. According to the volume of serum or plasma

$$\alpha\text{-HBDH (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.6 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank})$$

2. According to the protein concentration of sample

$$\alpha\text{-HBDH (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.6 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

3. According to the weight of sample

$$\alpha\text{-HBDH (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 0.6 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / W$$

4. According to the quantity of cell or bacteria

$$\alpha\text{-HBDH (U/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 0.6 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the concentration of standard, 300 μmol/L = 0.3 μmol/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;

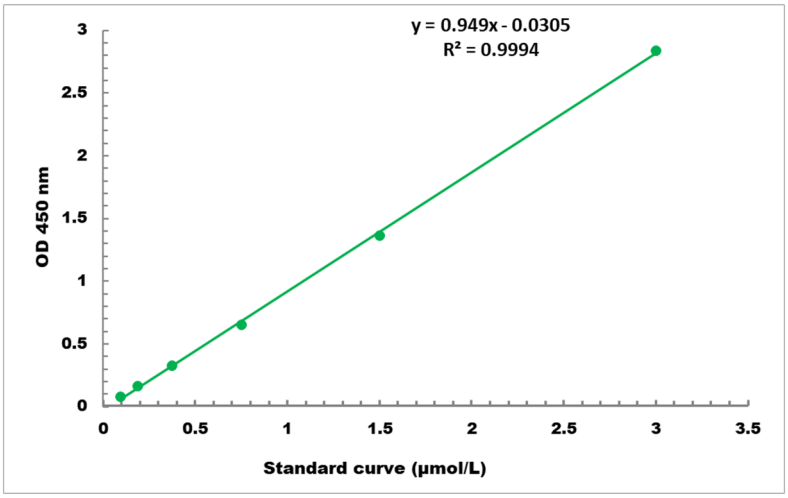
$V_{Assay}$: the volume of Assay buffer, 1 ml;

T: the reaction time, 5 minutes;

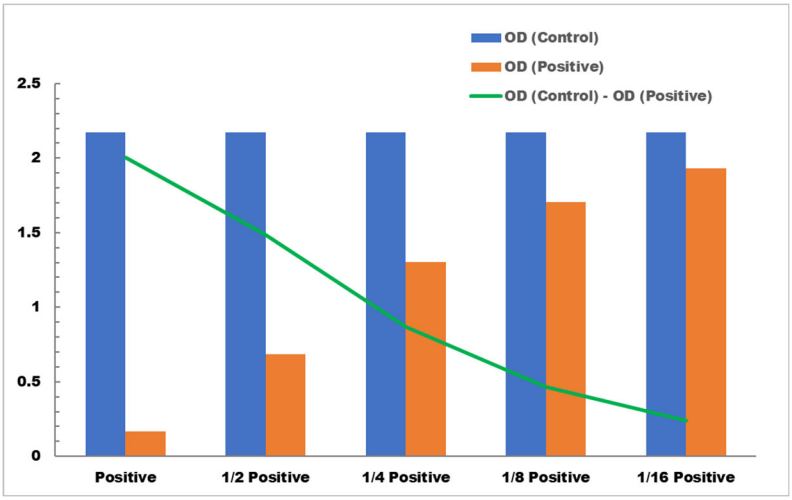
N: the quantity of cell or bacteria, $N \times 10^4$.

## 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