# alpha HBDH Activity Assay Kit

ARG83561 alpha HBDH Activity Assay Kit can be used to measure alpha HBDH in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, other biological fluids

Catalog number: ARG83561

Package: 100 tests

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## PRINCIPLE OF THE ASSAY

ARG83561 alpha HBDH Activity Assay Kit t 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.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store Positive Control and Substrate A at-20 °C, all other component at 2-8°C. Use the kit before expiration date.

| Component | Quantity | Storage |
|---|---|---|
| Microplate | 1 X 96-well plate | |
| Standard | 1 vial (lyophilized) | 4 °C |
| Positive Control | 1 vial (lyophilized) | -20 °C |
| Substrate A | 1 vial (lyophilized) | -20 °C |
| Substrate B | 1 ml | 4 °C |
| Reaction Buffer | 10 ml | 4 °C |
| Assay Buffer | 4 x 30 ml | 4 °C |
| Dye Reagent A | 1 vial (lyophilized) | 4 °C |
| Dye Reagent B | 1 vial (lyophilized) | 4 °C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and pipette tips
- Deionized or distilled water

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Positive Control and Substrate A at-20 °C, all other component at 2-8°C. Use the kit before expiration date.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and bacteria- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation. Mix and sonicate with 1 ml Assay buffer per $5 \times 10^6$ cell or bacteria. Centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue- 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.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

## REAGENT PREPARATION

- **Substrate A:** Reconstitute the **Substrate** A with **1 ml** of Reaction Buffer. Allow the **Substrate A** keep on bench for few minutes. Make sure the **Substrate A** is dissolved completely and mixed thoroughly before use.

- **Dye Reagent A:** Reconstitute the **Dye Reagent A** with **9 ml** of distilled water. Allow the **Dye Reagent A** keep on bench for few minutes. Make sure the **Dye Reagent A** is dissolved completely and mixed thoroughly before use

- **Positive Control:** Reconstitute the **Positive Control** with **100 µl** of distilled water. Allow the **Positive Control** keep on bench for few minutes. Make sure the **Positive Control** is dissolved completely and mixed thoroughly before use.

- **Standard:** Reconstitute the **Standard** with 1 ml of distilled water. Allow the **Standard** keep on bench for few minutes. Make sure the **Standard** is dissolved completely, then add 0.15 ml into 0.85 ml distilled water to yield 300 nmol/ml standard. Perform 2-fold serial dilutions of the top standards to make the standard curve.

## ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

Summary of alpha HBDH Activity Assay Kit Procedure

| 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 A | 10 µl | 10 µl | - | - | 10 µl |
| Substrate B | 10 µl | 10 µl | - | - | 10 µl |
| Distilled water | - | 10 µl | - | 100 µl | - |
| Mix well | | | | | |
| 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 well Incubate for 5 min at RT<br>Read the OD at 450 nm | | | | | |

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

a.) Definition: One unit of alpha HBDH activity is defined as the enzyme reduce 1 µmol NADH per minute.

$C_{Standard}$: the concentration of standard, 300 nmol/mL = 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, $N \times 10^4$.

b.) Calculation:

Formula:

a). According to the serum or plasma

alpha HBDH (U/ml) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / [(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T]$

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

b). According to the protein concentration

alpha HBDH (U/ml) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times C_{Protein}) \times T]$

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

c). According to the protein concentration

alpha HBDH (U/ml) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times C_{Protein}) \times T]$

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

d). According to the quantity of cell or bacteria

alpha HBDH ($U/10^4$) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times N / V_{Assay}) \times T]$

$= 2.5 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times N]$