Beta HB Assay Kit

ARG83566 beta HB Assay Kit can be used to measure beta HB in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids

Catalog number: ARG83566

Package: 100 tests

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PRINCIPLE OF THE ASSAY

ARG83566 Beta HB 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.

MATERIALS PROVIDED & STORAGE INFORMATION

Store Enzyme at-20 °C a, 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
Enzyme	1 vial (lyophilized)	-20 °C
Reaction Buffer	10 ml	4 °C
Assay Buffer	4x 30 ml	4 °C
Dye Reagent A	1 vial (lyophilized)	4 °C
Dye Reagent B	1 ml	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 Enzyme at-20 °C a, 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

Cell and bacteria- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 106 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.

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 EDTA or 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

- **Enzyme:** Reconstitute the **Enzyme** with **1 ml** of Reaction Buffer. Allow the **Enzyme** keep on bench for few minutes. Make sure the **Enzyme** 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 **Stand Dye Reagent A** 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 200µl **Reconstitute Standard** into 800µl **distilled water** to yield the **Working Standard**, the **Working Standard** concentration will be 5 µmol/mL

  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.

1. Add **80 µl Reaction Buffer** into all wells.
2. Add **10 µl Sample**, **Standard**, **Distilled water** into respective wells
3. Add **10 µl Enzyme** into all wells.
4. Mix well
5. Add **90 µl** Dye Reagent A into all wells.
6. Add **10 µl** Dye Reagent B into all wells.
7. Mix well, incubate at **RT** for **5 min.** Read the OD at 450nm.

Summary of Beta HB Assay Kit Procedure

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 well			
Working Standard	90 µl	90 µl	90 µl
Dye Reagent	10 µl	10 µl	10 µl
Mix well incubate for 5 min at RT. Read the OD at 450 nm			

CALCULATION OF RESULTS

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

a.) Definition:

$C_{Standard}$: the concentration of standard, 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, $N \times 10^4$.

b.) Calculation:

Formula:

a). According to the Volume:

beta HB (µmol/ml) = $(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times V_{Sample}]$

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

b). According to the weigh:

beta HB (μmol/g) = ($C_{Standard}$ x $V_{Standard}$) × (OD_{Sample}- OD_{Blank}) / [($OD_{Standard}$- OD_{Blank}) x (W × V_{Sample} / V_{Assay})]

= **5 x (OD_{Sample}- OD_{Blank}) / [($OD_{Standard}$- OD_{Blank}) x W]**

b). According to the Cells or bacteria:

beta HB (μmol/10^4) = ($C_{Standard}$ x $V_{Standard}$) × (OD_{Sample}- OD_{Blank}) / [($OD_{Standard}$- OD_{Blank}) x (N × V_{Sample} / V_{Assay})]

= **5 x (OD_{Sample}- OD_{Blank}) / [($OD_{Standard}$- OD_{Blank}) x N]**