Beta-Hydroxybutyrate Assay Kit

Catalog No.: abx295109

Size: 96 tests

Detection Range: 0.01 mmol/L – 2.00 mmol/L

Sensitivity: 0.01 mmol/L

Storage: Store all components at 4°C. Store Buffer Solution A, Enzyme Reagent, and Chromogenic Reagent in the dark.

Application: For detection and quantification of Beta-Hydroxybutyrate content in serum, plasma, urine, and tissue homogenates.

Introduction

Abbexa's Beta-Hydroxybutyrate Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Beta-Hydroxybutyrate content. Beta-Hydroxybutyrate is converted to acetoacetate, while concurrently NAD+ is reduced to NADH. NADH reduces the Chromogenic Reagent to produce a yellow-colored product with an absorbance maximum at 450 nm. The intensity of the color is proportional to the Beta-Hydroxybutyrate content, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution A: 2 × 50 ml
3. Enzyme Reagent: 2 Vials
4. Buffer Solution B: 10 ml
5. Chromogenic Reagent: 2 × 1.5 ml
6. Standard (10 mmol/L): 1 ml
7. Plate sealer: 2

Materials required but not provided

1. Microplate reader (450 nm)
2. Double distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. 50 kDa Ultrafiltration tubes
6. Centrifuge
7. Vortex mixer
8. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum, Plasma, Urine:** If sample is cloudy, centrifuge at 10,000 × g for 10 minutes. Centrifuge in a 50 kDa Ultrafiltration tube at 10,000 × g for 15 minutes. Take the supernatant, keep on ice, and assay immediately.
- **Tissue Homogenates:** Weigh approximately 20 mg of tissue and wash in cold PBS (0.01 M, pH 7.4). Homogenize in 180 µl double distilled water at 4 ºC. Centrifuge at 10,000 × g for 10 minutes, then take the supernatant and centrifuge in a 50 kDa Ultrafiltration tube at 10,000 × g for 15 minutes. Take the supernatant, keep on ice, and assay immediately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with double distilled water, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human Serum/Plasma	1
Rat Serum/Plasma	1
Mouse Serum/Plasma	1
10 % Rat Liver Tissue Homogenate	1
10 % Rat Kidney Tissue Homogenate	1
10 % Rat Heart Tissue Homogenate	1
Human Urine	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Enzyme Reagent:** Keep on ice during use.
- **Enzyme Working Solution:** Dissolve a vial of Enzyme Reagent with 1 ml of double distilled water and mix fully. Use immediately or store at -20 ºC in the dark for up to 1 month.
- **Enzyme Reaction Working Solution:** Prepare 50 µl per well by mixing Enzyme Working Solution with Buffer Solution B at a 1:4 ratio (10 µl Enzyme Working Solution + 40 µl Buffer Solution B per well). Prepare fresh before use and use immediately.
- **Chromogenic Reagent Working Solution:** Prepare 160 µl per well by mixing Chromogenic Reagent with Buffer Solution A at a 1:7 ratio (20 µl Chromogenic Reagent + 140 µl Buffer Solution A per well). Prepare fresh before use and use immediately.
- **Stock Standard Solution (5 mmol/L):** Dilute 150 µl of 10 mmol/L standard with 150 µl double distilled water and mix fully. Store at 4 °C for up to 2 days.
- **Standards:** Label 7 tubes with 2.0 mmol/L, 1.5 mmol/L, 1.2 mmol/L, 1.0 mmol/L, 0.8 mmol/L, 0.5 mmol/L, and 0.2 mmol/L. Prepare standard dilutions as summarized in the following table:

Standard Dilution (mmol/L)	2.0	1.5	1.2	1.0	0.8	0.5	0.2
5 mmol/L Standard (µl)	80	60	48	40	32	20	8
Buffer Solution A (µl)	120	140	152	160	168	180	192

For the blank, or 0 mmol/L standard, use pure Buffer Solution A. The volume of each standard will be 200 µl.

Note:

- Allow all reagents apart from the Enzyme Reagent to equilibrate to room temperature before use.

B. Assay Procedure

1. Add 10 µl of diluted Standard to the Standard wells.
2. Add 10 µl of Sample to the Sample wells.
3. Add 10 µl of Buffer Solution A to the Blank wells.
4. Add 50 µl of Enzyme Reaction Working Solution to each well and mix fully.
5. Incubate at 37 ºC for 10 minutes.
6. Add 160 µl Chromogenic Reagent Working Solution to each well and mix fully.
7. Incubate at 37 ºC for 30 minutes.
8. Measure the OD of each well with a microplate Reader at 450 nm

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Beta-Hydroxybutyrate in each sample well can be derived with the following formulae:

1. Serum, Plasma, Urine Samples:

$$\textbf{Beta} - \textbf{Hydroxybutyrate Content}\ (\textbf{mmol/L}) = \frac{(\Delta \boldsymbol{A} - \boldsymbol{b})}{\boldsymbol{a}} \times \boldsymbol{f}$$

2. Tissue samples:

$$\textbf{Beta} - \textbf{Hydroxybutyrate Content}\ (\textbf{mmol/L}) = \frac{(\Delta \boldsymbol{A} - \boldsymbol{b})}{\boldsymbol{a}} \times \frac{\boldsymbol{V}}{\boldsymbol{m}} \times \boldsymbol{f}$$

where:

ΔA	$OD_{Sample} - OD_{Blank}$
m	Weight of sample (0.1 g)
V	Volume of homogenate (0.9 ml)
f	Sample dilution factor