β-Hydroxybutyrate (Ketone Body) Assay Kit

Catalog No. 700190

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GENERAL INFORMATION

Materials Supplied

Catalog Number	Item	Quantity
700191	β-HB Assay Buffer	1 vial
700192	β-Hydroxybutyrate Standard	2 vials
700193	β-HB Enzyme Solution	2 vials
700194	β-HB Colorimetric Detector	2 vials
400014	96-Well Plate (Colorimetric Assay)	1 plate
400012	96-Well Cover Sheets	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbances between 445-455 nm
2. Adjustable pipettes and a repeat pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

β-Hydroxybutyrate (β-HB; 3-hydroxybutyric acid) is a "ketone body" which is produced in the liver, mainly from the oxidation of fatty acids, and is exported to peripheral tissues for use as an energy source. The term 'ketone body' refers to three molecules, acetoacetate, β-HB, and acetone. β-HB and acetoacetate transport energy from the liver to the other tissues and acetone is generated by spontaneous decarboxylation of acetoacetate.[1] The presence of ketosis may be normal or pathologic. Normally ketosis can indicate that lipid metabolism has been activated and the pathway of lipid degradation is intact. Normal ketosis is prevalent in many circumstances such as during fasting, after prolonged exercise or after a high fat diet. Pathological causes of ketosis include multiple organ failure, diabetes, childhood hypoglycemia, corticosteroid or growth hormone deficiency, intoxication with alcohol or salicylates and several inborn errors of metabolism.[2] In acutely ill patients, these ketone bodies can accumulate in the body to cause ketoacidosis, which leads to the potentially life threatening condition known as metabolic acidosis.[3] The presence and degree of ketosis can be determined by measuring blood levels of β-HB.

Ordinarily, β-HB accounts for approximately 75% of the ketone bodies in serum.[4-6] Measurement of β-HB provides a reliable index of the level of ketoacidosis, including the detection of subclinical ketosis.[7-9] In diabetics, β-HB measurements (and blood glucose) can be used for the assessment of the severity of diabetic coma and is essential for the exclusion of hyperosmolar non-ketotic diabetic coma. The measurement of β-HB is also used to monitor insulin requirements, based on existing hyperketonemia.[10] β-HB has more recently been evaluated for use in neurodegenerative diseases and inhibition of adipocyte lipolysis.[11-15]

About This Assay

Cayman's β-HB (Ketone Body) Assay Kit provides a simple, reproducible, and sensitive tool for measuring β-HB levels in plasma, serum, or urine. The method for β-HB determination is based upon the oxidation of D-3-Hydroxybutyrate to acetoacetate by the enzyme 3-hydroxybutyrate dehydrogenase.[16] Concomitant with this oxidation, the cofactor NAD^+ is reduced to NADH. In the presence of diaphorase, NADH reacts with the colorimetric detector WST-1 to produce a formazan dye with an absorbance maximum at 445-455 nm (see Figure 1 on page 6). The absorbance of the dye is directly proportional to the β-HB concentration.

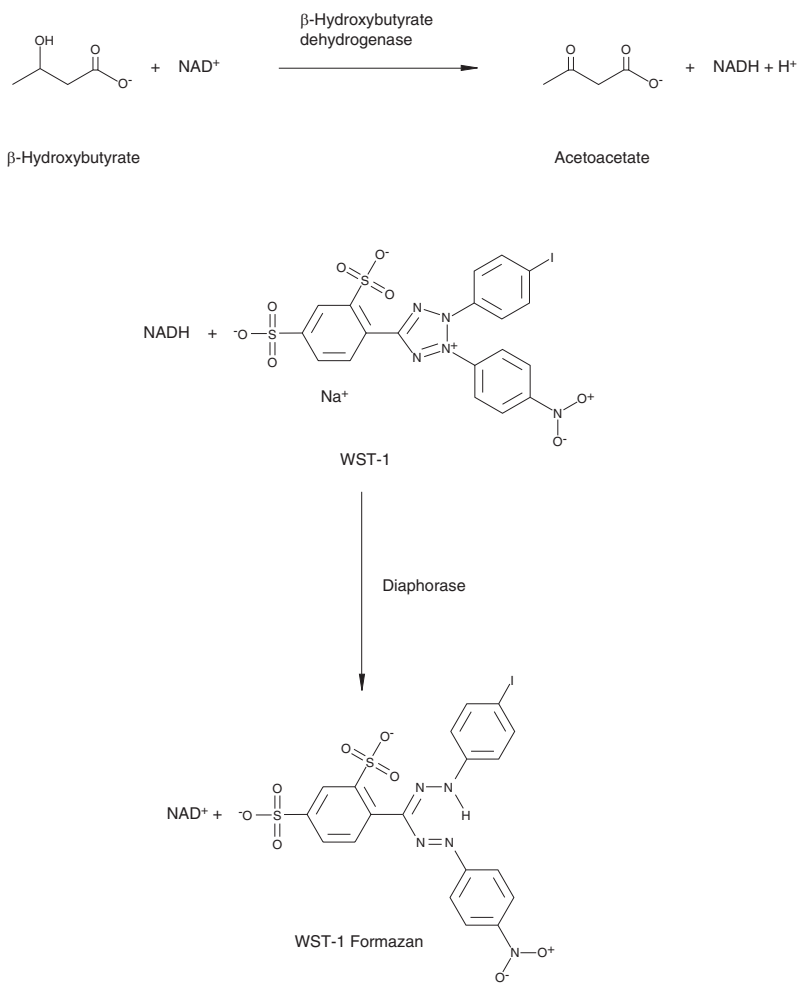

Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. β-HB Assay Buffer - (Catalog No. 700191)

The vial contains 25 ml of 100 mM Tris-HCl, pH 8.5. Thaw the Assay Buffer at room temperature. Once thawed, the Assay Buffer is ready to use in the assay and for diluting reagents and samples. When stored at -20°C, the thawed Assay Buffer is stable for at least six months.

2. β-Hydoxybutyrate Standard - (Catalog No. 700192)

Each vial contains a lyophilized powder of DL-Hydroxybutyrate. Reconstitute the contents of the vial with 1 ml of β-HB Assay Buffer (Catalog No. 700191). This reconstituted standard solution is used to prepare the β-HB standard curve. The reconstituted standard is stable for six hours on ice. *NOTE: When reconstituted, the solution will become a 1.0 mM D-Hydroxybutyrate solution.*

3. β-HB Enzyme Solution - (Catalog No. 700193)

Each vial contains a lyophilized enzyme mixture. Reconstitute the contents of the vial with 2.4 ml of β-HB Assay Buffer (Catalog No. 700191). The reconstituted enzyme solution is stable for two hours on ice. This enzyme solution is used to prepare the developer solution in step 5. One vial of the enzyme solution is sufficient to evaluate 48 wells.

4. β-HB Colorimetric Detector - (Catalog No. 700194)

Each vial contains 125 µl of WST-1 solution. It is ready to use as supplied. Thaw the detector solution and store on ice. The detector is also used to prepare the developer solution in step 5. One vial of the colorimetric detector is sufficient to evaluate 48 wells.

5. Developer Solution

Prepare the developer solution by adding 100 µl of the β-HB Colorimetric Detector (Catalog No. 700194) to the vial of the reconstituted Enzyme Solution (Catalog No. 700193). Store the developer solution on ice. The developer solution is stable for one hour.

Sample Preparation

β-HB concentrations in plasma and serum can vary over a rather wide range, with normal levels measuring 0.02-1.5 mM and increasing to as high as 3-5 mM in diabetics. β-HB concentrations in urine can be as high as 30-50 mM during diabetic ketoacidosis.[6,7,17,18]

Plasma

1. Collect blood using an anticoagulant such as heparin, EDTA, or citrate.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice. If not assaying the same day, freeze at -80°C. The plasma sample will be stable for one month while stored at -80°C.
3. Plasma should be diluted 1:5-1:10 with Assay Buffer before assaying.

Serum

1. Collect blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, freeze at -80°C. The serum sample will be stable for one month while stored at -80°C.
4. Serum should be diluted 1:5-1:10 with Assay Buffer before assaying

Urine

1. Collection of urine does not require any special treatment. If not assaying the same day, freeze at -80°C.
2. Urine should be diluted 1:10 with Assay Buffer before assaying.

NOTE: β-HB values from urine samples can be standardized using Cayman's Creatinine Assay Kit (Catalog No. 500701).

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of the β-HB standard curve and samples to be measured in triplicate is given in Figure 2. We suggest you record the contents of each well on the template sheet provided (see page 19).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	A	S1	S1	S1	S9	S9	S9	S17	S17	S17
B	B	B	B	S2	S2	S2	S10	S10	S10	S18	S18	S18
C	C	C	C	S3	S3	S3	S11	S11	S11	S19	S19	S19
D	D	D	D	S4	S4	S4	S12	S12	S12	S20	S20	S20
E	E	E	E	S5	S5	S5	S13	S13	S13	S21	S21	S21
F	F	F	F	S6	S6	S6	S14	S14	S14	S22	S22	S22
G	G	G	G	S7	S7	S7	S15	S15	S15	S23	S23	S23
H	H	H	H	S8	S8	S8	S16	S16	S16	S24	S24	S24

A-H = Standards
S1-S24 = Sample Wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps to maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 100 µl in all wells.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples be assayed at least in triplicate, but it is the user's discretion to do so.
- The assay is performed at 25°C.
- Monitor the absorbance at 445-455 nm.

Standard Preparation

Take eight clean test tubes and label them A-H. Add the amount of 1 mM β-HB standard solution and Assay Buffer to each tube as described in Table 1. We recommend that you store these diluted standards for no more than one to two hours.

Tube	β-HB Stock Solution (µl)	Assay Buffer (µl)	β-HB Concentration (mM)
A	0	200	0
B	5	195	0.025
C	10	190	0.05
D	20	180	0.1
E	40	160	0.2
F	60	140	0.3
G	80	120	0.4
H	100	100	0.5

Table 1. Preparation of β-Hydroxybutyrate standard curve

Performing the Assay

1. **β-Hydroxybutyrate Standard Wells** - Add 50 µl of each standard (tubes A-H) to two or three wells (see suggested plate configuration, Figure 2, page 9).
2. **Sample Wells** - Add 50 µl of the diluted samples to two or three wells.
3. Initiate the reaction by adding 50 µl of the Developer Solution to all wells being used.
4. Incubate the plate at 25°C in the dark for 30 minutes.
5. Read the absorbance at 445-455 nm using a plate reader.

ANALYSIS

Calculations

1. Calculate the average absorbance of each standard and sample.
2. Subtract the absorbance value of standard A (0 mM) from itself and all other values (both standards and samples). This is the corrected absorbance.
3. Plot the corrected absorbance values (from step 2 above) of each standard as a function of the final β-HB concentration (mM) (see Table 1, page 11). A typical β-HB standard curve is shown in Figure 3, on page 14.
4. Calculate the values of the β-HB samples using the equation obtained from the linear regression of the standard curve by substituting the corrected absorbance values for each sample into the equation.

$$\text{β-Hydroxybutyrate (mM)} = \left[\frac{\text{Corrected absorbance - (y-intercept)}}{\text{Slope}}\right] \text{x Dilution}$$

Performance Characteristics

Precision:

When a series of 48 human plasma and urine samples were assayed on the same day, the intra-assay coefficient of variation was 4.05% and 3.68%, respectively. When a series of 48 human plasma and urine samples were assayed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 3.18% and 3.03%, respectively.

Assay Range:

Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 0-0.5 mM β-HB.

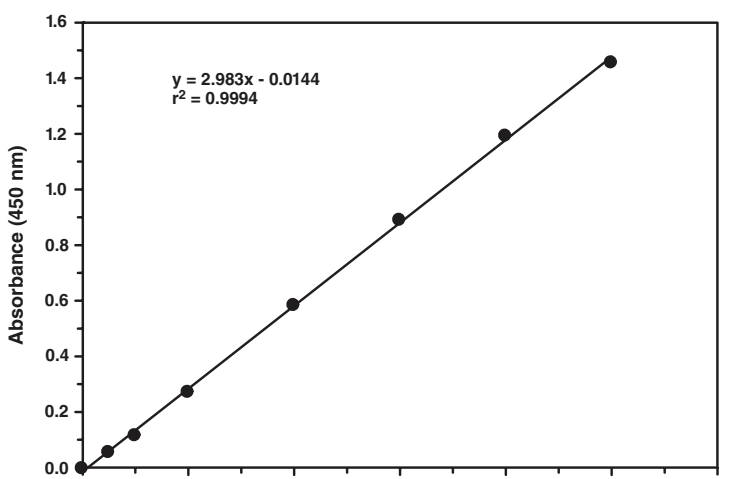

Figure 3. β-Hydroxybutyrate standard curve

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Carefully tap the side of the plate with your finger to remove bubbles B. Be careful not to splash the contents of the wells
No β-HB was detected in the sample	A. β-HB concentration was too low B. The sample was too dilute	Do not dilute samples and re-assay
Sample absorbance values are above highest point in standard curve	A. β-HB concentration was too high in the sample B. The sample was too concentrated	Dilute samples with assay buffer and re-assay. *NOTE: Remember to account for the dilution factor when calculating β-HB concentration.*

References

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Related Products

Adipogenesis Assay Kit - Cat. No. 10006908
Adipolysis Assay Kit - Cat. No. 10009381
ChREBP Cell-Based Translocation Assay Kit - Cat. No. 10010060
ChREBP Transcription Factor Assay Kit - Cat. No. 10006909
Cortisol EIA Kit - Cat. No. 582121
Cortisol Express EIA Kit - Cat. No. 10006791
DPP (IV) Inhibitor Screening Assay Kit - Cat. No. 700210
FAAH Inhibitor Screening Assay Kit - Cat. No. 10005196
FABP4 Inhibitor/Ligand Screening Assay Kit - Cat. No. 10010231
Ghrelin (human acylated) EIA Kit - Cat. No. 10006306
Ghrelin (human unacylated) EIA Kit - Cat. No. 10008952
Ghrelin (rat acylated) EIA Kit - Cat. No. 10006307
Ghrelin (rat unacylated) EIA Kit - Cat. No. 10008953
Glucose Assay Kit - Cat. No. 10009582
8-hydroxy-2-deoxy-Guanosine EIA Kit - Cat. No. 589320
Insulin (rat) EIA Kit - Cat. No. 589501
Leptin (human) EIA Kit - Cat. No. 500010
Liver X Receptor β Transcription Factor Assay Kit - Cat. No. 10011119
SREBP-1 Transcription Factor Assay Kit - Cat. No. 10010854
Steatosis Colorimetric Assay Kit - Cat. No. 10012643
Triglyceride Assay Kit - Cat. No. 10010303

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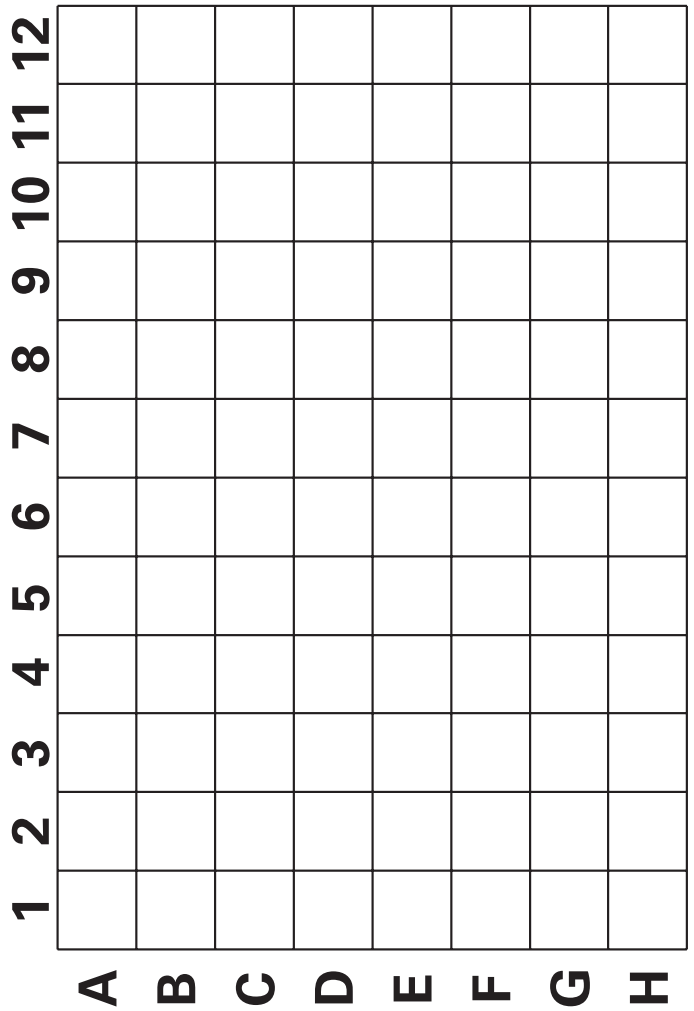
Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



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