Ketone Body Assay Kit (Colorimetric)

Ketone Body Assay Kit is an assay kit for the quantification of Ketone Body (acetoacetate and 3-hydroxybutyrate) in Serum, plasma and urine.

Catalog number: ARG82173

Package: 200 tests
(100 tests of acetoacetic acid (AcAc) and 100 tests of 3-hydroxybutyric acid (B-OHB) in 96 well plates)

TABLE OF CONTENTS

SECTION **Page**

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INTRODUCTION

Ketone bodies (acetoacetate, beta-hydroxybutyrate, and the spontaneous breakdown product of acetoacetate, acetone) are the water-soluble molecules containing the ketone group that are produced by the liver from fatty acids during periods of low food intake (fasting), carbohydrate restrictive diets, starvation, prolonged intense exercise, alcoholism or in untreated (or inadequately treated) type 1 diabetes mellitus. Ketone bodies are readily transported into tissues outside the liver and converted into acetyl-CoA, which then enters the citric acid cycle and is oxidized in the mitochondria for energy. In the brain, ketone bodies are also used to make acetyl-CoA into long-chain fatty acids.

In normal individuals, there is a constant production of ketone bodies by the liver and their utilization by extrahepatic tissues. The concentration of ketone bodies in blood is maintained around 1 mg/dl. Their excretion in urine is very low and undetectable by routine urine tests (Rothera's test).

When the rate of synthesis of ketone bodies exceeds the rate of utilization, their concentration in blood increases; this is known as ketonemia. This is followed by ketonuria – excretion of ketone bodies in urine. The overall picture of ketonemia and ketonuria is commonly referred as ketosis. The smell of acetoacetate and/or acetone in breath is a common feature in ketosis.

When a type 1 diabetic suffers acute biological stress (infection, heart attack, or physical trauma), or fails to administer enough insulin they may enter the pathological state of diabetic ketoacidosis. Under these circumstances, the low or absent insulin levels in the blood, combined with the inappropriately high

glucagon concentrations, induce the liver to produce glucose at an inappropriately increased rate, causing acetyl-CoA resulting from the beta-oxidation of fatty acids, to be converted into ketone bodies. The resulting very high levels of ketone bodies lower the pH of the blood plasma which reflexively triggers the kidneys to excrete a very acid urine. The high levels of glucose and ketones in the blood also spill, passively, into the urine (the ability of the renal tubules to reabsorb glucose and ketones from the tubular fluid, being overwhelmed by the high volumes of these substances being filtered into the tubular fluid). The resulting osmotic diuresis of glucose causes the removal of water and electrolytes from the blood resulting in potentially fatal dehydration. [Wikipedia]

PRINCIPLE OF THE ASSAY

In this kit, acetoacetate (AcAc) and beta-hydroxybutyrate (B-OHB) levels are determined using an enzymatic assay based on 3-hydroxybutyrate dehydrogenase (HBDH) catalyzed reactions, in which the change in NADH absorbance measured at 340 nm is directly related to both AcAc and BOH concentrations. The assay kit is a enzymatic method to detect NADH by absorbance at 340 nm and it is proportionate to AcAc or BOH activity. The total incubation time is 15-20 min only.

$$\text{acetoacetate} + \text{NADH} + \text{H}^+ \underset{\text{pH 9.5}}{\overset{\text{pH 7.0}}{\rightleftharpoons}} \text{beta-hydroxybutyrate} + \text{NAD}^+$$

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
AcAc Assay Buffer	20 ml	-20°C
AcAc Reagent	1 vial (lyophilized)	-20°C
AcAc Standard (80 mM)	200 µl	-20°C
B-OHB Assay Buffer	20 ml	-20°C
B-OHB Reagent	1 ml	-20°C
B-OHB Standard (80 mM)	200 µl	-20°C
HBDH Enzyme	120 µl	-20°C

The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt, 3 weeks after reconstitution. This kit is sufficient for up to 100 assays of 3-hydroxybutyric acid and up to 100 assays of acetoacetic acid in 96 well plates (for around 50 samples with each assay).

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 340 nm
- Clear flat bottomed 96-well microplate
- 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 the kit at -20°C at all times.
- *During experiment, keep HBDH enzyme on ice or in refrigerator (2-8°C) before use. Return the enzyme to -20°C immediately after used.*
- Briefly spin down the reagents before use.
- 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.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Collect serum and assay immediately or aliquot and store samples at ≤ -80°C for up to 30 days. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -80°C for up to 30 days. Avoid repeated freeze-thaw cycles.

Urine- Collect the urine by micturating directly into a sterile container. Remove

impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at -80°C for up to 30 days.

Samples should be clear and free of particles or precipitates. Avoid using haemolytic, icteric or lipaemic samples.

REAGENT PREPARATION

- AcAc Reagent: Reconstitute the AcAc Reagent with 1 ml of distilled water to yield an AcAc Reagent concentration of 10 mM. Unused AcAc Reagent is stable for three weeks when stored frozen at -20°C.
- AcAc Standard: Prepare 8 mM standard by mixing 5 µl of AcAc stock standard (80 mM) with 45 µL of distilled water (1:10 dilution).
- AcAc Working Reagent: *For wells contain distilled water (blank), Standard and assayed Sample. Prepare before use,* for each well, mix 195 µl of AcAc assay Buffer, 8 µl of reconstituted AcAc Reagent and 0.5 µl of HBDH Enzyme for each well.
- AcAc Sample Blank Working Reagent: *For sample blank well. Prepare before use,* for each well, mix 195 µl of AcAc assay Buffer, 8 µl of reconstituted AcAc Reagent and 0.5 µl of distilled water (no enzyme) for each well.
- B-OHB Standard: Prepare 8 mM standard by mixing 5 µl of B-OHB stock standard (80 mM) with 45 µL of distilled water (1:10 dilution).
- B-OHB Working Reagent: *For wells contain distilled water (blank), Standard and assayed Sample*. *Prepare before use,* for each well, mix 195 µl of B-OHB assay Buffer, 8 µl of B-OHB Reagent and 0.5 µl of HBDH

Enzyme for each well.

- B-OHB Sample Blank Working Reagent: *For sample blank well*. *Prepare before use,* for each well, mix 195 µl of B-OHB assay Buffer, 8 µl of B-OHB Reagent and 0.5 µl of distilled water (no enzyme) for each well.

ASSAY PROCEDURE

All reagents (except enzyme), microplate and spectrophotometer should be equilibrated to room temperature before use. *During experiment, keep HBDH enzyme on ice or in refrigerator (2-8°C) before use. Return the enzyme to -20°C immediately after used.*

For AcAc Assay:

1. Add 5 µl of distilled water into blank wells of a clear, flat-bottom, 96-well plate.
2. Add 5 µl of 8 mM AcAc standard into standard wells.
3. Add 5 µl of each sample into two wells. One is for sample to assay, the other one is for sample blank well.
4. Add 195 µl of AcAc Working Reagent into the wells for Blank (distilled water only), standard and Sample to assay.
5. Add 195 µl of AcAc Sample Blank Working Reagent into the Sample Blank wells.
6. Gently tap the plate to mix it well immediately. Incubation the plate at room temperature for 5 min.
7. Read O.D. with a microplate reader at 340 nm.

For B-OHB Assay:

1. Add **5 µl** of **distilled water** in blank wells of a clear, flat-bottom, 96-well plate.
2. Add **5 µl** of **8 mM B-OHB standard** into standard wells.
3. Add **5 µl** of **each sample into two wells**. One is for sample to assay, the other one is for sample blank well.
4. Add **195 µl** of **B-OHB Working Reagent** into the wells for Blank (distilled water only), standard and Sample to assay.
5. Add **195 µl** of **B-OHB Sample Blank Working Reagent** into the Sample Blank wells.
6. Gently tap the plate to mix it well immediately. Incubation the plate at **room temperature for 15 min**.
7. **Read O.D.** with a microplate reader at **340 nm**.

Summary of Ketone Body Assay Procedure

Reagent	Blank	Standard	Sample blank	Sample (assayed)
Distilled water	5 µl	-	-	-
Standard (AcAc or B-OHB)	-	5 µl	-	-
Sample	-	-	5 µl	5 µl
AcAc or B-OHB Working Reagent	195 µl	195 µl	-	195 µl
AcAc or B-OHB Sample Blank Working Reagent	-	-	195 µl	-
Gently tap the plate to mix it well immediately				
AcAc assay: Incubation the plate at **room temperature for 5 min**. **B-OHB assay:** Incubation the plate at **room temperature for 15 min**.				
Read the OD with a microplate reader at **340 nm** immediately.				

CALCULATION OF RESULTS

1. Calculate the acetoacetic acid (AcAc) concentration from the OD values of the distilled water, 8 mM Standard, Sample and Sample Blank wells:

   $[AcAc]\ (mM) = 8 \times (OD_{Sample\ Blank} - OD_{Sample}) / (OD_{H2O} - OD_{Standard})$

   Note: OD data are from AcAc assay.

2. Calculate the 3-hydroxybutyric acid (B-OHB) concentration from the OD values of the distilled water, 8 mM Standard, Sample and Sample Blank wells:

   $[B\text{-}OHB]\ (mM) = 8 \times (OD_{Sample} - OD_{Sample\ Blank}) / (OD_{Standard} - OD_{H2O})$

   Note: OD data are from B-OHB assay.

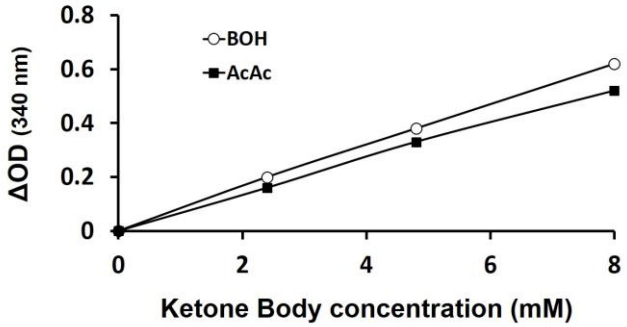
3. Total ketone body (TKB) concentration is calculated as:

   $[Total\ ketone\ body]\ (mM) = [AcAc] + [B\text{-}OHB]$

4. If the calculated [AcAc] or [B-OHB] is higher than 8mM, dilute sample with distilled water and repeat assay. Multiple the results by the dilution factor.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and this kit does not need serial diluted standards.



QUALITY ASSURANCE

Sensitivity

Linear detection range of 0.12 to 8 mM for each ketone body. The mean MDD was 0.12 mM.