

# Ketone body Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Ultraviolet spectrophotometer/Microplate reader

**Catalog Number:** BC###5

**Size:** 100T/96S

## Components:

Reagent	Size	Storage
Reagent I A	Solution 20 mL×1	4°C
Reagent I B	Solution 20 mL×1	4°C
Reagent II A	Powder×2	-20°C
Reagent II B	Powder×2	-20°C
Reagent III	Powder×2	-20°C
Standard A	Powder×1	4°C
Standard B	Powder×1	-20°C

## Solution preparation:

1. Reagent II A: Take one powder and add 600  $\mu$ L distilled water before use. Mix thoroughly. Unused reagents should be store at -20°C for three weeks. Avoid repeated freezing and thawing.
2. Reagent II B: Take one powder and add 300  $\mu$ L distilled water before use. Mix thoroughly. Unused reagents should be store at -20°C for three weeks. Avoid repeated freezing and thawing.
3. Reagent III: Take one powder and add 400  $\mu$ L distilled water before use(100T/96S). Mix thoroughly. Unused reagents should be store at -20°C. Avoid repeated freezing and thawing. Reagent III is not easy to save, so give one more powder.
4. Standard A: 8 mg 3-hydroxybutyric acid (BOH). Add 980  $\mu$ L distilled water before use. Mix thoroughly. That is 8 mg/mL of BOH standard solution. Dilute with distilled water to 0.2mg/mL standard solution before use, record as Standard solution A.
5. Standard B: 8 mg acetoacetic acid (AcAc). Add 950  $\mu$ L distilled water before use. Mix thoroughly. That is 8mg/mL of AcAc standard solution. Dilute with distilled water to 0.05mg/mL standard solution before use, record as Standard solution B.
6. BOH Working Solution: According to the ratio of 85:4:1, Reagent I A, Reagent II A and Reagent III are mixed into working solution before use. According to the test requirements. Mix thoroughly. Keep it at 37°C for 15 min (this step can't be omitted). The working solution should be used up in 4 hours.
7. AcAc Working Solution: According to the ratio of 87:2:1, Reagent I B, Reagent II B and Reagent III are mixed into working solution before use. According to the test requirements. Mix thoroughly. Keep it at 37°C for 15 min (this step can't be omitted). The working solution should be used up in 4 hours.

## Product Description :

Ketone bodies are intermediate products of fatty acid oxidative decomposition in liver. It includes

Acetoacetic acid (AcAc) and  $\beta$ - Hydroxybutyric acid (BOH) and acetone. The amount of acetone in ketone body is very small, and it is absorbed immediately. AcAc and BOH is oxidized in extrahepatic tissue through blood flow. The citric acid cycle provides more energy for those tissues, such as bone, myocardium and renal cortex.

At pH 7.0 and 37°C,  $\beta$ - Hydroxybutyrate dehydrogenase (HBDH) catalyzes the dehydrogenation of BOH to produce phthalic acid, and  $\text{NAD}^+$  is reduced to NADH. At pH 8.8 and 37°C, HBDH reduced AcAc to 3-hydroxybutyrate or decarboxylated to acetone, and NADH was oxidized to  $\text{NAD}^+$ . NADPH has a characteristic absorption peak at 340nm. The content of BOH and AcAc can be calculated by detecting the change of absorbance at 340nm. Then the content of ketone body in the sample can be calculated.

### **Reagents and Equipment Required but Not Provided:**

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, pipette, micro quartz cuvette/96 well UV flat -bottom plate, mortar/homogenizer, ice and distilled water.

### **Procedure**

#### **I. Sample preparation:**

Serum (plasma), urine or other liquid samples: direct determination.

#### **II. Determination procedure:**

1. Preheat ultraviolet spectrophotometer/microplate reader for 30 min, adjust wavelength to 340 nm, set zero with distilled water.

2. Determination of BOH content:

(1) Blank tube: Add 20  $\mu\text{L}$  distilled water, 180  $\mu\text{L}$  BOH Working Solution in the micro quartz cuvette or 96 well UV flat-bottom plate. Mix them immediately and time them. Record the absorbance value at 20s  $A_{\text{BOH B1}}$ . Reaction for 5min at 37°C. Record the absorbance value at 5min20s  $A_{\text{BOH B2}}$ . Calculation  $\Delta A_{\text{BOH B}} = A_{\text{BOH B2}} - A_{\text{BOH B1}}$ .

(2) Standard tube: Add 20  $\mu\text{L}$  Standard solution A, 180  $\mu\text{L}$  BOH Working Solution in the micro quartz cuvette or 96 well UV flat-bottom plate. Mix them immediately and time them. Record the absorbance value at 20s  $A_{\text{BOH ST1}}$ . Reaction for 5min at 37°C. Record the absorbance value at 5min20s  $A_{\text{BOH ST2}}$ . Calculation  $\Delta A_{\text{BOH ST}} = A_{\text{BOH ST2}} - A_{\text{BOH ST1}}$ .

(3) Test tube: Add 20  $\mu\text{L}$  Sample, 180  $\mu\text{L}$  BOH Working Solution in the micro quartz cuvette or 96 well UV flat-bottom plate. Mix them immediately and time them. Record the absorbance value at 20s  $A_{\text{BOH SA1}}$ . Reaction for 5min at 37°C. Record the absorbance value at 5min20s  $A_{\text{BOH SA2}}$ . Calculation  $\Delta A_{\text{BOH SA}} = A_{\text{BOH SA2}} - A_{\text{BOH SA1}}$ .

3. Determination of AcAc content:

(1) Blank tube: Add 20  $\mu\text{L}$  distilled water, 180  $\mu\text{L}$  AcAc Working Solution in the micro quartz cuvette or 96 well UV flat-bottom plate. Mix them immediately and time them. Record the absorbance value at 20s  $A_{\text{AcAc B1}}$ . Reaction for 5min at 37°C. Record the absorbance value at 5min20s  $A_{\text{AcAc B2}}$ . Calculation  $\Delta A_{\text{AcAc B}} = A_{\text{AcAc B1}} - A_{\text{AcAc B2}}$ .

(2) Standard tube: Add 20  $\mu\text{L}$  Standard solution A, 180  $\mu\text{L}$  AcAc Working Solution in the micro quartz cuvette or 96 well UV flat-bottom plate. Mix them immediately and time them. Record the absorbance

value at 20s  $A_{AcAc\ ST1}$ . Reaction for 5min at 37°C. Record the absorbance value at 5min20s  $A_{AcAc\ ST2}$ .

Calculation  $\Delta A_{AcAc\ ST} = A_{AcAc\ ST1} - A_{AcAc\ ST2}$ .

(3) Test tube: Add 20  $\mu$ L Sample, 180  $\mu$ L AcAc Working Solution in the micro quartz cuvette or 96 well UV flat-bottom plate. Mix them immediately and time them. Record the absorbance value at 20s  $A_{AcAc\ SA1}$ . Reaction for 5min at 37°C. Record the absorbance value at 5min20s  $A_{AcAc\ SA2}$ . Calculation  $\Delta A_{AcAc\ SA} = A_{AcAc\ SA1} - A_{AcAc\ SA2}$ .

Note: blank tube and standard tube only need to be test one or two times.

### III. Calculations:

#### 1. BOH Calculate

$$\begin{aligned} \text{BOH content } (\mu\text{mol/mL}) &= (\Delta A_{\text{BOH SA}} - \Delta A_{\text{BOH B}}) \div (\Delta A_{\text{BOH ST}} - \Delta A_{\text{BOH B}}) \times C_{\text{BOH}} \div 126.09 \times 1000 \\ &= 1.586 \times (\Delta A_{\text{BOH SA}} - \Delta A_{\text{BOH B}}) \div (\Delta A_{\text{BOH ST}} - \Delta A_{\text{BOH B}}) \end{aligned}$$

#### 2. AcAc Calculate

$$\begin{aligned} \text{AcAc content } (\mu\text{mol/mL}) &= (\Delta A_{\text{AcAc SA}} - \Delta A_{\text{AcAc B}}) \div (\Delta A_{\text{AcAc ST}} - \Delta A_{\text{AcAc B}}) \times C_{\text{AcAc}} \div 108.02 \times 1000 \\ &= 0.463 \times (\Delta A_{\text{AcAc SA}} - \Delta A_{\text{AcAc B}}) \div (\Delta A_{\text{AcAc ST}} - \Delta A_{\text{AcAc B}}) \end{aligned}$$

#### 3. Ketone body Calculate

Ketone body content ( $\mu$ mol/mL) = BOH content + AcAc content

$C_{\text{BOH}}$ : Concentration of Standard solution A, 0.2mg/mL;

$C_{\text{AcAc}}$ : Concentration of Standard solution B, 0.05mg/mL.

126.09: Molecular weight of BOH

108.02: Molecular weight of AcAc

1000: 1mmol=1000 $\mu$ mol

### Note:

1. If the measured absorbance value  $A > 1.5$  or  $\Delta A > 0.2$ , it is recommended to dilute the sample before measuring, and multiply the dilution factor in the calculation formula; if the measured absorbance value is low or close to the blank OD value, it is recommended to increase the sample volume before performing the measurement.

### Related products

BC0710/BC0715  $\alpha$ -Ketoglutarate Dehydrogenase( $\alpha$ -KGDH) Activity Assay Kit

BC2150/BC2155 Citric Acid (CA) Content Assay Kit

BC0950/BC0955 Succinate Dehydrogenase (SDH) Activity Assay Kit

BC0380/BC0385 Pyruvate Dehydrogenase (PDH) Activity Assay Kit

BC2160/BC2165 Isocitrate Dehydrogenase Mitochondrial (ICDHm) Activity Assay Kit