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Ketone body Assay Kit

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

Operation Equipment: Ultraviolet spectrophotometer/ Microplate reader

Catalog Number: AK0003

Size: 100T/96S

Components:

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

Solution preparation:

1. Reagent II A: Take one powder and add 600 μL distilled water before use. Mix thoroughly. Unused reagents should be store at -20℃ for three weeks. Avoid repeated freezing and thawing.

2. Reagent II B: Take one powder and add 300 μL distilled water before use. Mix thoroughly. Unused reagents should be store at -20℃ for three weeks. Avoid repeated freezing and thawing.

3. Reagent III: Take one powder and add 600 μL distilled water before use(100T/96S). Mix thoroughly. Unused reagents should be store at -20℃ . Avoid repeated freezing and thawing. Reagent III is not easy to save, so give one more powder.

4. 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℃ for 15 min (this step can't be omitted). The working solution should be used up in 4 hours.

5. 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℃ for 15 min (this step can't be omitted). The working solution should be used up in 4 hours.

6. Standard A: 8 mg 3-hydroxybutyric acid (BOH). Add 960 μL distilled water before use. Mix thoroughly. That is 80 μmol/mL of BOH standard solution. Dilute with distilled water to 2000 nmol/mL standard solution before use, record as Standard solution A.

7. Standard B: 8 mg acetoacetic acid (AcAc). Add 980 μL distilled water before use. Mix thoroughly. That is 80 μmol/mL of AcAc standard solution. Dilute with distilled water to 500 nmol/mL standard solution before use, record as Standard solution B.

Product Description：

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

Acetoacetic acid (AcAc) and β- 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℃, β- Hydroxybutyrate dehydrogenase (HBDH) catalyzes the dehydrogenation of BOH to produce phthalic acid, and NAD^+ is reduced to NADH. At pH 8.8 and 37℃, HBDH reduced AcAc to 3-hydroxybutyrate or decarboxylated to acetone, and NADH was oxidized to 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 μL distilled water, 180 μL 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 $A_{BOH\ B1}$. Reaction for 5min at 37℃. Record the absorbance value at 5min20s $A_{BOH\ B2}$. Calculation $\Delta A_{BOH\ B}= A_{BOH\ B2}- A_{BOH\ B1}$.

(2) Standard tube: Add 20 μL Standard solution A, 180 μL 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 $A_{BOH\ ST1}$. Reaction for 5min at 37℃. Record the absorbance value at 5min20s $A_{BOH\ ST2}$. Calculation $\Delta A_{BOH\ ST}= A_{BOH\ ST2}- A_{BOH\ ST1}$.

(3) Test tube: Add 20 μL Sample, 180 μL 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 $A_{BOH\ SA1}$. Reaction for 5min at 37℃. Record the absorbance value at 5min20s $A_{BOH\ SA2}$. Calculation $\Delta A_{BOH\ SA}= A_{BOH\ SA2}- A_{BOH\ SA1}$.

3. Determination of AcAc content:

(1) Blank tube: Add 20 μL distilled water, 180 μL 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 $A_{AcAc\ B1}$. Reaction for 5min at 37℃. Record the absorbance value at 5min20s $A_{AcAc\ B2}$. Calculation $\Delta A_{AcAc\ B}= A_{AcAc\ B1}- A_{AcAc\ B2}$.

(2) Standard tube: Add 20 μL Standard solution A, 180 μL 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℃ . 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 μL Sample, 180 μL 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℃ . 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

BOH content (nmol/mL) = $(\Delta A_{BOH\ SA}-\Delta A_{BOH\ B}) \div (\Delta A_{BOH\ ST}-\Delta A_{BOH\ B}) \times C_{BOH}$

2. AcAc Calculate

AcAc content (nmol/mL) = $(\Delta A_{AcAc\ SA}-\Delta A_{AcAc\ B}) \div (\Delta A_{AcAc\ ST}-\Delta A_{AcAc\ B}) \times C_{AcAc}$

3. Ketone body Calculate

Ketone body content (nmol/mL)= BOH content + AcAc content

C_{BOH} : Concentration of Standard solution A, 2000nmol/mL;

C_{AcAc} : Concentration of Standard solution B, 500nmol/mL.

Note:

1. If the measured absorbance value A>1.5 or ΔA>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

AK0282/AK0281 α-Ketoglutarate Dehydrogenase(α-KGDH) Activity Assay Kit

AK0400/AK0399 Citric Acid (CA) Content Assay Kit

AK0504/AK0503 Succinate Dehydrogenase (SDH) Activity Assay Kit

AK0554/AK0553 Pyruvate Dehydrogenase (PDH) Activity Assay Kit

AK0249/AK0248 Isocitrate Dehydrogenase Mitochondrial (ICDHm) Activity Assay Kit