Acetoacetate（AcAc）Content Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC5070

Size: 50T/24S

Components:

Reagent	Size	Storage
Extract solution	Solution 60 mL×1	4℃
Reagent I	Solution 70 mL×1	4℃
Reagent II	Powder×2	-20℃
Reagent III	Powder×2	-20℃
Chromogenic solution	Solution 4mL×1	-20℃
Standard	Powder×1	-20℃

Solution preparation:

1. Reagent II: Take one powder and add 1.5mL distilled water before use. Mix thoroughly. Unused reagents should be store at -20℃ for three weeks. Avoid repeated freezing and thawing.
2. Reagent III: Take one powder and add 400μL distilled water before use (about 40T). Mix thoroughly. Unused reagents should be store at -20℃ for two weeks. Avoid repeated freezing and thawing. Reagent III is not easy to save, so give one more powder.
3. Working Solution: According to the ratio of 85:4:1, Reagent I, Reagent II 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**.
4. Standard: lithium acetoacetate. Add 980μL distilled water before use. Mix thoroughly. That is 8mg/mL of lithium acetoacetate standard solution.

Product Description：

Acetoacetic acid (AcAc) is one of the important components of ketone body. It is about 20% of the total ketone body in normal people. It is a strong organic acid produced by oxidation of fatty acids. Normal content of acetoacetic acid is harmless to human body. In diabetic patients, the amount of acetoacetic acid is accumulated due to the decrease of carbohydrate use or starvation due to the metabolic disorder of sugar. Acetoacetic acid can be converted into acetone as well as acetone β-Hydroxybutyric acid.

At pH 7.0 and 37℃, β- Hydroxybutyrate dehydrogenase (HBDH) reduced AcAc to 3-hydroxybutyrate or decarboxylated to acetone, and NADH was oxidized to NAD^+. In the presence of 1-mPMS, WST-1 can react with NADH to produce water-soluble formazan with a characteristic absorption peak at 450nm. The content of AcAc can be calculated by detecting the wavelength change at 450nm.

Reagents and Equipment Required but Not Provided：

Spectrophotometer, desk centrifuge, pipette, 1mL glass cuvette, mortar/homogenizer, ice and distilled water.

Procedure

I. Sample preparation:

A. Tissue

It is suggested to take about 0.1g of tissue and add 1mL of Extract solution. Fully grinding on ice, centrifuge at 12000g for 10 minutes at 4℃, the supernatant is used for test.

B. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, the supernatant is discarded after centrifugation. It is suggested to take about 5 million bacteria/cell and add 1mL of Extract solution. Bacteria and cell should be broken by ultrasonication (Power: 20%, work time 3s, interval 10s, repeat for 30 times). Centrifuge at 12000g for 10 minutes at 4℃, the supernatant is used for test.

C. Serum (plasma) or other liquid samples: Detect sample directly.

II. Determination procedure：

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 450nm, set zero with distilled water.

2. Dilute 8mg/mL lithium acetoacetate standard solution with distilled water to0.25、0.2、0.15、0.1、0.05、0.025、0.0125、0.00625 mg/mL standard solution before use.

3. Determination:

Reagent（μL）	Test tube	Contrast tube	Blank tube	Standard tube
Sample	100	100		
Distilled water			100	
Standard solution				100
Working solution	900		900	900
Reagent III		900		
React at 37℃ for 10min.				
Chromogenic solution	50	50	50	50
React at 37℃ for 20min. (Light avoidance)				
Measure absorbance at 450nm. Record as A_T、A_C、A_B、A_S。$\Delta A_T=A_B-(A_T-A_C)$, $\Delta A_S=A_B-A_S$。				

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

III. Calculations：

1. Standard curve

Take the concentration of each standard solution as x-axis, and the corresponding ΔA standard is y-axis. Then the linear regression equation y＝kx+b is obtained. Bring ΔA into the equation to get x (μmol/mL).

2. Calculate

(1) Calculate by protein concentration

AcAc content (μmol/mg prot)=$x\times V_S\div(V_S\times Cpr)\div108.02\times1000=9.258x\div Cpr$

(2) Calculate by sample weight

AcAc content (μmol/g weight)=$x \times V_S \div (W \times V_S \div V_E) \div 108.02 \times 1000 = 9.258x \div W$

(3) Calculate by number of cells

AcAc content (μmol/10^4 cell)=$x \times V_S \div (cell \times V_S \div V_E) \div 108.02 \times 1000 = 9.258x \div cell$

(4) Calculate by volume

AcAc content (μmol/mL)=$x \times V_S \div V_S \div 108.02 = 9.258x$

V_S: Sample volume, 100μL=0.1mL;

V_E: Extract solution volume, 1mL;

W: Sample weight, g;

Cpr: Protein concentration of the sample, mg/mL;

cell: Total number of bacteria or cells,10^4.

108.02：Molecular weight of AcAc

Note:

1. After color development, please complete the test within 10 minutes.
2. If the measured absorbance value is lower or higher than the linear range absorbance value. The sample can be added or diluted before determination.

Examples:

1. Take 100μL bovine serum to test, follow the determination procedure to operate. Determination with 1mL glass cuvette, and calculate $\Delta A_T = A_B - (A_T - A_C) = 1.101 - (10129 - 0.063) = 0.035$, standard curve: y=0.299x+0.006, calculate x=0.097, according with mass of sample to calculate:
AcAc content (μmol/mL) =0.9258x=0.898μmol/mL.

Related products

BC0710/BC0715 α-Ketoglutarate Dehydrogenase(α-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