Acetoacetate（AcAc）Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC5075

Size: 100T/48S

Components:

Reagent	Size	Storage
Extract solution	Solution 110 mL×1	2-8℃
Reagent I	Solution 25 mL×1	2-8℃
Reagent II	Powder×2	-20℃
Reagent III	Powder×2	-20℃
Chromogenic solution	Solution 1.5 mL×1	-20℃
Standard	Powder×1	-20℃

Solution preparation:

1. **Reagent II:** Add 600μL distilled water to each Reagent II before use. Mix thoroughly. It can be stored at -20℃ for three weeks after dispensing to avoid repeated freezing and thawing.
2. **Reagent III:** Add 400μL distilled water to each Reagent III before use. Mix thoroughly. It can be stored at -20℃ for two weeks after dispensing to avoid repeated freezing and thawing. Duplicate Reagent III is provided for its instability.
3. **Working Solution:** Reagent I, Reagent II and Reagent III are mixed by the ratio of 170μL:8μL:2μL（1T）to make working solution according to sample number. 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 950μL distilled water before use. Mix thoroughly. The 8 mg/mL of lithium acetoacetate standard solution could be stored at -20℃ for four weeks.

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.

In the assay, acetoacetate is reacted by a coupling enzyme to produce a product with a 450 nm absorption peak, proportional to the acetoacetate present..

Reagents and Equipment Required but Not Provided：

Spectrophotometer/microplate reader, desk centrifuge, pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer/cell ultrasonic crusher, 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 200W, 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/microplate reader for 30min, adjust the wavelength to 450nm, and set spectrophotometer counter to zero with distilled water.

2. Standard working solution: Dilute 8mg/mL lithium acetoacetate standard solution with distilled water to 0.25, 0.2, 0.15, 0.1, 0.05, 0.025, 0.0125, 0.00625mg/mL standard solution before use.

3. Determination:

Reagent（μL）	Test tube	Contrast tube	Blank tube	Standard tube
Sample	20	20	-	-
Distilled water	-	-	20	-
Standard solution	-	-	-	20
Working solution	180	-	180	180
Reagent I	-	180	-	-
React at 37℃ for 10min.				
Chromogenic solution	10	10	10	10
React at 37℃ for 20min. (Light avoidance)				
Take 200μL to 96 well flat bottom plate or micro glass cuvette. Measure absorbance at 450nm. Record as A_T、A_C、A_B、A_S. $\Delta A_T=A_B-(A_T-A_C)$, $\Delta A_S=A_B-A_S$. Blank tube and standard curve only need to be tested one or twice.				

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_T into the equation to get x (mg/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)=$x\times V_S\div(W\times V_S\div V_E)\times1000\div108.02=9.258x\div W$

(3) Calculate by number of cells

AcAc content (μmol/10^4 cell)=x×V_S÷(N×V_S÷V_E) ×1000÷108.02=9.258x÷N

(4) Calculate by volume

AcAc content (μmol/mL)=x×V_S÷V_S÷108.02×1000=9.258x

V_S: Sample volume, 20μL=0.02mL;

V_E: Extract solution volume, 1mL;

W: Sample weight, g;

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

N: Total number of bacteria or cells,10^4;

108.02: Relative molecular mass of lithium acetoacetate, mg/mmol;

1000: Unit conversion factor, 1 mmol=1000 μmol.

Note:

1. After color development, please complete the test within 15 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 20μL bovine serum to test, follow the determination procedure to operate. Determination with 96 well flat-bottom plate, and calculate $\Delta A_T=A_B-(A_T-A_C)=0.802-(0.837-0.076)=0.041$, standard curve: y=0.2259x+0.0145, calculate x=0.117, according with volume of sample to calculate:

AcAc content (μmol/mL) =9.258x=1.083μmol/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