ATP-Citrate Lyase (ACL) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0098

Size: 50T/48S

Components:

Extract solution I: Liquid 50 mL×1, store at 4°C;

Extract solution II: Liquid 0.5mL×1, store at-20°C;

Reagent I: Liquid 60 mL×1, store at 4°C;

Reagent II: Powder×1, store at -20°C . Add 1mL of Reagent I when the solution will be used. The rest of reagent store at -20°C;

Reagent III: Powder×1, store at -20°C . Add 5mL of Reagent I when the solution will be used. The rest of reagent store at -20°C;

Reagent IV: Powder×1, store at -20°C . Add 1mL of Reagent I when the solution will be used. The rest of reagent store at -20°C;

Reagent V: Liquid 30μL×1, store at 4°C . Add 1mL of Reagent I when the solution will be used. The rest of reagent store at -20°C;

Preparation of extraction reagent: Prepare according to the ratio of Extract solution I: Extract solution II = 990: 10 (V: V). Prepare according to the sample number. It is forbidden to add Reagent II to the Reagent I all at once.

Product Description:

ATP-citrate lyase is a key cytosolic enzyme that catalyzes the production of acetyl-CoA from citric acid. Produced acetyl-CoA is the main raw material for the synthesis of fatty substances such as fatty acids and cholesterol, and can participate in the modification of related important proteins. It is a pivotal substance for energy substance metabolism in the body.

In the presence of ATP and coenzyme A, ACL can catalyze the cleavage of citric acid into acetyl coenzyme A, oxaloacetate, ADP, and phosphate. Malate dehydrogenase further catalyzes oxaloacetate and NADH to produce malate and NAD^+, leading light absorption decreases at 340 nm.

Required but Not Provided:

Spectrophotometer, desk centrifuge, water-bath, balance, transferpettor, mortar/homogenizer, 1 mL quartz cuvette, ice and distilled water.

Protocol

I. Preparation:

1. Cells or bacterial

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Number of cells or bacteria (10^4): volume of extraction reagent (mL) is 500 ~ 1000: 1. It is suggested to take about 5 million bacteria/cell and add 1mL extraction reagent. Bacteria/cell is split by ultrasonication (power 20%, ultrasonic 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 and g 4℃ for 10 minutes. Take the supernatant on ice for test.

2. Tissue:

Mass of tissue (g): the volume of the extraction reagent (mL) is 1: 5 ~ 10, Add 1 mL of extraction reagent to 0.1 g of tissue. Homogenate on ice. Centrifuge at 8000 g and 4℃ for 10 minutes. Take the supernatant on ice for test.

3. Serum: detect directly.

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer for 30 minutes, adjust wavelength to 340 nm, set the counter to zero with distilled water.

2. Preheat reagent I in 37℃ (mammal) or 25℃ (other species) for 10 minutes.

3. Operation table:

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)
Reagent I	805	805
Reagent II	20	20
Reagent III	100	100
Reagent IV	20	20
Reagent V	5	5
Sample	50	-
Water	-	50

The above reagents are added into the 1 mL quartz cuvette in sequence. Mix thoroughly. The initial absorbance A1 for 10s and the absorbance A2 for 130s after reaction 2 minutes are recorded at 340 nm, and keep the reaction temperature at 37℃ (mammal) or 25℃ (other species) as far as possible. $\Delta A_B=A1_B-A2_B$. $\Delta A_T=A1_T-A2_T$. $\Delta A=\Delta A_T-\Delta A_B$. Blank tube need only be done once.

Note: You can use reagent I: reagent II: reagent III: reagent IV: reagent V = 161: 4: 20: 4: 1 to prepare a working solution according the sample number, prepared the working solution will be used.

III. ACL Calculation:

1. Serum

Unit definition: One unit of enzyme is defined as the amount of enzyme that that catalyzes the consumes of 1 nmol of NADH per minute every mL of serum in the reaction system.

ACL (U/mL) $=\Delta A\div(\varepsilon\times d)\times V_{RT}\div V_{SA}\div T\times10^9=1607.7\times\Delta A$

2. Tissue

1) Protein concentration:

Unit definition: One unit of enzyme is defined as the amount of enzyme that that catalyzes the consumes of 1 nmol of NADH per minute every mg tissue protein in the reaction system.

ACL (U/mg prot) $=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(Cpr\times V_{SA})\div T\times10^9=1607.7\times\Delta A\div Cpr$

2) Sample weight:

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Unit definition: One unit of enzyme is defined as the amount of enzyme that that catalyzes the consumes of 1 nmol of NADH per minute every gram of tissue weight in the reaction system.

ACL (U/g weight) $=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(W\div V_E\times V_{SA})\div T\times 10^9=1607.7\times\Delta A\div W$

3. Cells or bacteria

Unit definition: One unit of enzyme is defined as the amount of enzyme that that catalyzes the consumes of 1 nmol of NADH per minute every 10^4 cells or bacteria in the reaction system.

ACL (U/10^4 cell) $=\Delta A\div(\varepsilon\times d)\times V_{RT}\div(500\times V_{SA}\div V_E)\div T\times 10^9=3.215\times\Delta A$

ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;
d: Light path of cuvette, 1 cm;
V_{RT} : Total reaction volume, 0.001 L;
V_{SA} : Sample volume, 0.05 mL;
V_E : Extract solution volume, 1 mL;
500: Cells or germ, 5million;
T: Reaction time, 2 minutes;
Cpr: Protein concentration, mg/mL; Protein concentration self-determined.

Experimental examples:

1. Take 0.1 g of ryegrass and add 1 ml of extraction reagent for sample processing. After centrifugation to take the supernatant, proceed according to the determination procedure. Calculate $\Delta A_T=A1_T-A2_T=1.549-1.512=0.037$, $\Delta A_B=A1_B-A2_B=0.411-0.41=0.001$, $\Delta A=\Delta A_T-\Delta A_B=0.037-0.001=0.036$. The enzyme activity is calculated according to the sample mass.

ACL (U/g weight) $=1607.7\times\Delta A\div W=578.772$ U/g weight.

2. Take 0.1 g of liver tissue and add 1 ml of extraction reagent for sample processing. After centrifugation to take the supernatant, proceed according to the determination procedure. Calculate $\Delta A_T=A1_T-A2_T=1.165-0.985=0.179$, $\Delta A_B=A1_B-A2_B=0.411-0.41=0.001$, $\Delta A=\Delta A_T-\Delta A_B=0.179-0.001=0.178$.

The enzyme activity is calculated according to the sample mass.

ACL (U/g weight) $=1607.7\times\Delta A\div W=2861.706$ U/g weight.

Related products:

AK0269/AK0268 Acetaldehyde Dehydrogenase(ALDH) Activity Assay Kit
AK0323/AK0322 Acetyl CoA carboxylase(ACC) Activity Assay Kit
AK0327/AK0326 Total Cholesterol(TC) Content Assay Kit