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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/ microplate reader

Cat No: AK0097

Size: 100T/96S

Components:

Extract solution I: Liquid 100 mL×1, store at 4℃;

Extract solution II: Liquid 1mL×1, store at-20℃;

Reagent I: Liquid 30 mL×1, store at 4℃;

Reagent II: Powder×1, store at -20℃ . Add 0.5mL of Reagent I when the solution will be used. The rest of reagent store at -20℃;

Reagent III: Powder×1, store at -20℃ . Add 2mL of Reagent I when the solution will be used. The rest of reagent store at -20℃;

Reagent IV: Powder×1, store at -20℃ . Add 0.5mL of Reagent I when the solution will be used. The rest of reagent store at -20℃;

Reagent V: Liquid 15μL×1, store at 4℃ . Add 0.5mL of Reagent I when the solution will be used. The rest of reagent store at -20℃;

Preparation of extraction liquid: 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/ microplate reader, desk centrifuge, water-bath, balance, transferpettor, mortar/ homogenizer, micro quartz cuvette/ 96 well flat-bottom plate (UV), ice and distilled water.

Protocol

I. Preparation:

1. Cells or bacterial

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/ microplate reader 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	161	161
Reagent II	4	4
Reagent III	20	20
Reagent IV	4	4
Reagent V	1	1
Sample	10	-
Water	-	10

The above reagents are added into the micro quartz cuvette/ 96-well plate (UV) 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 wavelength, 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:

a. micro quartz cuvette:

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) $=\Delta A\div(\varepsilon\times d)\times V_{RT}\div V_{SA}\div T\times 10^9=1607.7\times\Delta A$

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) =ΔA÷(ε×d) ×V_{RT}÷(Cpr×V_{SA}) ÷T×10^9=1607.7×ΔA÷Cpr

2) Sample weight:

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) =ΔA÷(ε×d) ×V_{RT}÷(W÷V_E ×V_{SA}) ÷T×10^9=1607.7×ΔA÷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) =ΔA÷(ε×d) ×V_{RT}÷(500×V_{SA}÷V_E) ÷T×10^9=3.215×ΔA

ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;

d: Light path of cuvette, 1 cm;

V_{RT} : Total reaction volume, 0.0002 L;

V_{SA} : Sample volume, 0.01 mL;

V_E : Extract solution volume, 1 mL;

500: Cells or germ, 5million;

T: Reaction time, 2 minutes;

Cpr: Protein concentration, mg/mL;

b. 96-well plate:

Change the Light path d- 1cm in the above formula to d-6cm (the light path of 96-well plate) for calculation.

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. After determination with micro quartz cuvette, calculate $\Delta A_T=A1_T-A2_T$ =1.7569- 1.7034=0.0535， $\Delta A_B=A1_B-A2_B$=0.459-0.457=0.002，ΔA=$\Delta A_T-\Delta A_B$=0.0535-0.002=0.0515. The enzyme activity is calculated according to the sample mass.

ACL (U/g weight) =1607.7×ΔA÷W=827.9655 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. After determination with micro quartz cuvette, calculate $\Delta A_T=A1_T-A2_T$ =1.2341- 1.0503=0. 1838， $\Delta A_B=A1_B-A2_B$=0.459-0.457=0.002，ΔA=$\Delta A_T-\Delta A_B$=0. 1838-0.002=0. 1818. The enzyme activity is calculated according to the sample mass.

ACL (U/g weight) =1607.7×ΔA÷W=2922.7986 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