ATP-Citrate Lyase (ACL) Activity Assay Kit

Note: The reagents have been changed, please be aware of and follow this instruction strictly.

Operation Equipment: Spectrophotometer

Cat No: BC4240

Size: 50T/48S

Components:

Extract solution I: Liquid 60 mL×1, store at 2-8℃.

Extract solution II: Liquid 0.6mL×1, store at-20℃; As a volatile reagent, it should be sealed as soon as possible after use and stored at -20°C.

Reagent I: Liquid 60 mL×1, store at 2-8℃;

Reagent II: Powder×1, store at -20℃. Before use, take one and add 1.3mL mL Reagent I to fully dissolve it. The unused reagent can be stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing.

Reagent III: Powder×1, store at -20℃. Before use, take one and add 6 mL Reagent I to fully dissolve it. The unused reagent can be stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing.

Reagent IV: Powder×1, store at -20℃. Before use, take one and add 1 mL Reagent I to fully dissolve it. The unused reagent can be stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing.

Reagent V: Powder×1, store at -20℃. Before use, take one and add 0.5 mL distilled water to fully dissolve it. The unused reagent can be stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing.

Reagent V-Working Solution: According to the sample number, according to the ratio of reagent V: distilled water = 0.025mL: 0.8mL (total 0.825mL, about 16T), the preparation should use up on the same day;

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:

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com

Spectrophotometer, Desk Centrifuge, Water-Bath/Constant Temperature Incubator, Balance, Transferpettor, Mortar/Homogenizer/Cell Ultrasonic Crusher, 1 mL Quartz Cuvette, Ice, 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 300W, ultrasonic 3s, interval 7s, repeat for 18times). Centrifuge at 8000 and g 4℃ for 10 minutes. Take the supernatant on ice for test.

2. Tissue:

Homogenize in an ice bath at a ratio of mass (g): volume of extract (mL) of 1:5~10 (it is recommended to weigh about 0.1g and add 1mL of extract). Centrifuge at 8000g for 10min, take the supernatant and put it on ice for measurement.

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℃ for 10 minutes.

3. Operation table: (Add the following reagents sequentially to a 1mL quartz cuvette)

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)
Reagent I	760	760
Reagent II	20	20
Reagent III	100	100
Reagent IV	20	20
Reagent V-Working Solution	50	50
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℃. $\Delta A_B=A1_B-A2_B$. $\Delta A_T=A1_T-A2_T$. $\Delta A=\Delta A_T-\Delta A_B$. Blank tube need only be done once.

Note: You can use reagent I: reagent II: reagent III: reagent IV: Reagent V-Working Solution = 760: 20: 100: 20: 50 to prepare a working solution according the sample number, prepared the working solution will be used.

III. ACL Calculation:

1. 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

2. 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}÷(N×V_{SA}÷V_E) ÷T×10^9=1607.7×ΔA÷N

3. Liquid

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) =ΔA÷(ε×d) ×V_{RT}÷V_{SA}÷T×10^9=1607.7×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, 0.001 L;

V_{SA}: Sample volume, 0.05 mL;

V_E: Extract solution volume, 1 mL;

N: Number of cells, in millions;

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 ΔA_T= $A1_T$-$A2_T$=1.549-1.512=0.037 , ΔA_B=$A1_B$-$A2_B$=0.411-0.41=0.001 , ΔA=ΔA_T-ΔA_B=0.037-0.001= 0.036. The enzyme activity is calculated according to the sample mass.

ACL (U/g weight) =1607.7×ΔA÷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 ΔA_T= $A1_T$-$A2_T$ =1.165-0.985=0.179 , ΔA_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×ΔA÷W=2861.706 U/g weight.

Related products:

BC0750/BC0755	Acetaldehyde Dehydrogenase(ALDH) Activity Assay Kit
BC6020/BC6025	Acetyl CoA carboxylase(ACC) Activity Assay Kit（Enzymatic method）
BC1980/BC1985	Total Cholesterol(TC) Content Assay Kit