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/ microplate reader

Cat No: BC4245

Size: 100T/96S

Components:

Extract solution I: Liquid 110 mL×1, store at 2-8℃;

Extract solution II: Liquid 0.6mL×2, store at-20℃; It is a volatile reagent, which should be sealed as soon as possible after use.

Reagent I: Liquid 30 mL×1, store at 2-8℃;

Reagent II: Powder×1, store at -20℃. Before use, take one and add 0.5 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 2.25 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 0.5 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 Reagent I 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 82T), 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:

Spectrophotometer/Microplate Reader, Desk Centrifuge, Water-Bath/Constant Temperature Incubator, Balance, Transferpettor, Mortar/ Homogenizer/Cell Ultrasonic Crusher, 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 200W, ultrasonic 3s, interval 7s, repeat for 18 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 spectrophotometer 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 micro quartz cuvette / 96-well UV plate)

Reagent (μL)	Test tube (A_T)	Blank tube (A_B)
Reagent I	152	152
Reagent II	4	4
Reagent III	20	20
Reagent IV	4	4
Reagent V-Working Solution	10	10
Sample	10	-
Water	-	10

Add the above reagents into the micro-quartz cuvette/96-well UV plate. Add the above reagents into the micro-quartz cuvette/96-well UV plate, mix well and then measure the absorbance value A1 at 340nm for 10s, quickly put it in 37℃ water bath or incubator for 2min (the temperature can be adjusted to 37℃ if the enzyme marker has temperature control function), and then quickly wiped dry to measure the absorbance value at 130s. A2, calculate ΔAt = A1t - A2t, ΔAb= A1b- A2b, ΔA = ΔAt - ΔAb (blank tube should be done only 1-2 times).

Note: You can use reagent I: reagent II: reagent III: reagent IV: Reagent V-Working Solution =

152: 4: 20: 4: 10 to prepare a working solution according the sample number, prepared the working solution will be used.

III. ACL Calculation:

a. micro quartz cuvette:

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, 0.0002 L;

V_{SA}: Sample volume, 0.01 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.

b. 96-well plate:

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=2679.5×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=2679.5×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=2679.5×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=2679.5×ΔA

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

d: Light path of cuvette, 0.6 cm;

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

V_{SA}: Sample volume, 0.01 mL;

V_E: Extract solution volume, 1 mL;

N: Number of cells, in millions;

T: Reaction time, 2 minutes;

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 ΔA_T=$A1_T$-$A2_T$ =1.7569-1.7034=0.0535，ΔA_B=$A1_B$-$A2_B$ =0.459-0.457=0.002，ΔA=ΔA_T-ΔA_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 ΔA_T=$A1_T$-$A2_T$ =1.2341-1.0503=0.1838，ΔA_B=$A1_B$-$A2_B$=0.459-0.457=0.002，ΔA=ΔA_T-ΔA_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：

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