Acetyl CoA carboxylase (ACC) Activity Assay Kit （Enzymatic method）

Detection Equipment: Spectrophotometer/microplate reader

Catalog Number: BC6020

Size: 50T/48S

Components:Please carefully check the volume of the reagent and the volume in the bottle before use. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation condition
Extract I	Liquid 110 mL×1	2-8℃ storage
Extract II	Liquid 0.6 mL×1	-20℃ storage
Reagent I A	Liquid 6 mL×1	2-8℃ storage
Reagent I B	Powder ×1	-20℃ storage
Reagent II	Powder ×1	-20℃ storage
Reagent III	Liquid 25 μL×1	2-8℃ storage
Reagent IV	Liquid 10 μL×1	2-8℃ storage
Reagent V	Powder ×1	-20℃ storage
Reagent VI	Powder ×1	-20℃ storage

Solution reparation :

1. Extract II: Volatile reagent, sealed as soon as possible after use, storage at -20℃.
2. Preparation of extraction solution: Extract I: Extract II =990μL: 10μL, according to the sample size of the mixture of immediate use, do not add Extract II to Extract I.
3. Reagent I: Before use, pour Reagent I B into Reagent I A, fully dissolve and wait for use; It can be storage at -20℃ for 4 weeks after subassembly to avoid repeated freezing and thawing.
4. Reagent II: Put the reagent in a brown bottle, add 5mL distilled water before use, and dissolve it fully before use; It can be storage at -20℃ for 4 weeks after subassembly to avoid repeated freezing and thawing.
5. Reagent III diluent: centrifugal before use, according to the sample size according to Reagent III: distilled water =1μL: 50μL (a total of 51μL, about 5T) fully mixed, ready for use.
6. Reagent IV diluent: centrifugal before use, according to the sample size according to Reagent IV: distilled water =1μL: 125μL (a total of 126μL, about 12T) fully mixed, ready for use.
7. Reagent V: Put the reagent in a brown bottle, add 5mL distilled water before use; It can be storage at -20℃ for 4 weeks after subassembly to avoid repeated freezing and thawing.
8. Reagent VI: The reagent is placed in a brown bottle, and 5mL distilled water is added before use. It can be storage at -20℃ for 4 weeks after subassembly to avoid repeated freezing and thawing.
9. Preparation of working liquid: Before clinical use, it is prepared according to the sample size according to the ratio of Reagent III diluent: Reagent IV diluent: Reagent V: Reagent VI =50μL:50μL:200μL: 200μL (a total of 500μL, about 5T).

Description:

Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA in vivo, and is A key enzyme in the synthesis of fatty acids and many secondary metabolites. The activity of ACC determines the synthesis speed and oil content of fatty acids to some extent.

ACC can catalyze acetyl-CoA, NaHCO3 and ATP to produce malonyl-CoA, ADP and inorganic phosphorus, and pyruvate kinase and lactate dehydrogenase further catalyze NADH to produce NAD+. The activity of ACC can be reflected by determining the NADH decline rate at 340nm.

$$\text{Acetyl CoA} + NaHCO_3 + \text{ATP} \xrightarrow{\text{Acetyl CoA}} \text{Malonyl CoA} + \text{ADP} + \text{Pi}$$

$$\text{Phosphoenolpyruvic Acid} + \text{ADP} \xrightarrow{\text{Pyruvate}} \text{Pyruvic Acid} + \text{ATP}$$

$$\text{Pyruvic Acid} + \text{NADH (340nm)} \xrightarrow{\text{Lactate}} \text{Lactate} + NAD^+$$

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorption value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, analytical balance, low temperature centrifuge, micro glass cuvette/96 well flat-bottom plate, adjustable pipette gun, mortar/homogenizer/cell ultrasonic crusher, whirlpool shaker, water bath/constant temperature incubator, distilled water and ice.

Protocol:

I. Sample processing (The sample size to be tested can be appropriately adjusted, and the specific proportion can be referred to the literature)

1. **Tissue:** According to the ratio of tissue mass (g) : extraction liquid volume (mL) = 1:5-10 (it is recommended to weigh about 0.1g tissue and add 1mL extract), homogenize in an ice bath, then centrifuge at 10000g at 4℃ for 10min, and take the supernatant and place it on ice for testing.
2. **Bacteria or cells:** Collect bacteria or cells into the centrifuge tube, discard the supernatant after centrifugation; According to the number of bacteria or cells (10^6) : the ratio of the volume of the extraction liquid (mL) = 5-10:1 (it is recommended that 5 million cells be added into 1mL of the extraction solution), the cells were broken by ultrasound in an ice bath (power 300W, ultrasonic 3 s, interval 7 s, total time 3 min). Then the samples were centrifuged at 10000g for 10min at 4℃, and the supernatant was removed and placed on ice until measured.
3. **Serum (plasma) :** Direct determination.

II. Measurement Steps

1. The spectrophotometer/microplate reader is preheated for more than 30min, the wavelength is adjusted to 340nm, and zero with the distilled water.
2. Preheat Reagent I and Working liquid at 37℃ for 5min according to the sample size before clinical use.
3. Operation table: (Add the following reagents in turn into micro glass cuvette/96 well flat-bottom plate)

Reagent name（μL）	Test tube	Blank tube
Reagent I	50	50
Sample	10	-
Distilled water	-	10
Working solution	100	100
Reagent II	40	40

The above reagents were added into micro glass cuvette/96 well flat-bottom plate in sequence, immediately and thoroughly mixed at 340nm to determine the absorption value A1 at 10s, quickly placed at 37℃ for accurate reaction for 10min(The enzyme marker has the temperature control function to adjust the temperature to 37℃), and quickly wiped dry to determine the absorption value A2 at 10min10s. Calculation $\Delta A_{text}=A1_{text}-A2_{text}$, $\Delta A_{blank}=A1_{blank}-A2_{blank}$, $\Delta A=\Delta A_{text}-\Delta A_{blank}$, the blank tube only needs to be done 1-2 times.

III. Calculations

1. The calculation formula for determination with 96-well UV plate is as follows:

1）Calculated by sample protein concentration:

Enzyme activity definition:Each mg of hiprotein catalyzes the conversion of 1nmol NADH to 1nmol NAD+ as an enzyme activity unit per minute in the reaction system.

ACC enzyme activity（U/mg prot）=$[\Delta A\times V_{total\ reaction}\div(\varepsilon\times d)\times10^9]\div(Cpr\times V_{sample})\div T=535.9\times\Delta A\div Cpr$

2）Calculated by sample quality:

Enzyme activity definition:Each g of tissue catalyzed the conversion of 1nmol NADH to 1nmol NAD+ as an enzyme activity unit per minute in the reaction system.

ACC enzyme activity（U/g mass）=$[\Delta A\times V_{total\ reaction}\div(\varepsilon\times d)\times10^9]\div(W\times V_{sample}\div V_{total\ sample})\div T=535.9\times\Delta A\div W$

3）Calculated by the number of bacteria or cells:

Enzyme activity definition:Each 10^6 bacteria or cells catalyzed the conversion of 1nmol NADH to 1nmol NAD+ as one unit of enzyme activity per minute in the reaction system.

ACC enzyme activity（U/10^6 cell）=$[\Delta A\times V_{total\ reaction}\div(\varepsilon\times d)\times10^9]\div(N\times V_{sample}\div V_{total\ sample})\div T=535.9\times\Delta A\div N$

4）Calculated by serum (pulp) volume:

Enzyme activity definition:Each mL of liquid catalyzes the conversion of 1nmol NADH to 1nmol NAD+ as an enzyme activity unit per minute in the reaction system.

ACC enzyme activity（U/mL）=$[\Delta A\times V_{total\ reaction}\div(\varepsilon\times d)\times10^9]\div V_{sample}\div T=535.9\times\Delta A$

$V_{total\ reaction}$: Total volume of the reaction system, 2×10^{-4}L;

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

d: Light diameter of 96 well UV plate light diameter, 0.6cm;

V_{sample}: Add the sample volume, 0.01mL;

$V_{total\ sample}$: Add extraction liquid volume, 1mL;

T: Reaction time, 10min;

Cpr: Sample protein concentration, mg/mL, self-determination of protein concentration;

W: Sample quality, g;

N: The number of bacteria or cells, measured in 10^6;

10^9: Unit conversion factor, 1mol=10^9nmol.

2. The calculation formula for the determination with trace quartz cuphor is as follows:

Change d=0.6cm in the above formula to d=1cm (microquartz cuphor light diameter) for calculation.

Note:

1. The temperature of the reaction liquid in the colorimetric dish must be kept at 37℃. Put a small beaker into a certain amount of 37℃ distilled water and put the beaker into the 37℃ water bath.
2. It is best for two people to do this experiment at the same time, one person to compare colors, one person to time, to ensure the accuracy of the experimental results.
3. When $A1_{text}$ <$A1_{blank}$, it is recommended to dilute the sample with distilled water and then determine; When the sample ΔA is too small, it is recommended to increase the sample size or extend the reaction time, and pay attention to the simultaneous modification of the calculation formula.
4. Since Extraction I contains protein (about 1mg/mL), if the protein concentration of the sample needs to be determined, the protein concentration of the extraction solution itself needs to be subtracted.

Experimental example:

1. Take 0.1044g mouse brain tissue was taken,Add 1mL of extract, homogenize, centrifuge, take supernatant, and then follow the measurement steps to measure and calculate with 96-well UV plate:ΔA=（$A1_{text}$-$A2_{text}$）-（$A1_{blank}$-$A2_{blank}$）=（0.828-0.162）-（0.788-0.774）=0.652，enzyme activity according to sample mass:
   ACC enzyme activity（U/g mass）=535.9×ΔA÷W=3346.8 U/g mass。
2. Take 0.1041g sunflower leaf tissue,Add 1mL of extract, homogenize, centrifuge, take supernatant, and then follow the measurement steps to measure and calculate with 96-well UV plate:ΔA=（$A1_{text}$-$A2_{text}$）-（$A1_{blank}$-$A2_{blank}$）=（0.841-0.811）-（0.788-0.774）=0.016，enzyme activity according to sample mass:
   ACC enzyme activity（U/g mass）=535.9×ΔA÷W=82.37 U/g mass。
3. Take 3×106 Raw cells,Add 1mL of extract, homogenize, centrifuge, take supernatant, and then follow the measurement steps to measure and calculate with 96-well UV plate:ΔA=（$A1_{text}$-$A2_{text}$）-（$A1_{blank}$-$A2_{blank}$）=（0.655-0.557）-（0.788-0.774）=0.084，enzyme viability according to cell number:
   ACC enzyme activity（U/10^6 cell）=535.9×ΔA÷N=15.01 U/10^6 cell。
4. Take 30μL rabbit serum, follow the measurement procedure, use 96 well UV plate to measure ΔA= (A1 determination -A2 determination) - (A1 blank -A2 blank) = (0.846-0.810) - (0.788-0.774) =0.022, calculate the enzyme activity according to the serum (plasma) volume of the sample:
   ACC enzyme activity（U/mL）=[ΔA×$V_{total\ reaction}$÷（ε×d）×10^9]÷V_{sample}÷T=178.63Δ×A=3.93U/mL

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