Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

CheKine™ Micro Acetyl CoA Carboxylase (ACC) Activity Assay Kit

Cat #: KTB1261 Size: 48 T/96 T

	CheKine™ Micro Acetyl CoA Carboxylase (ACC) Activity Assay Kit		
REF	**Cat #:** KTB1261	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and plant tissues, bacteria and cells, serum (plasma) or other biological fluids.		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

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. ACC activity determines, in part, the rate of fatty acid synthesis and the level of oil content. CheKine™ Micro Acetyl CoA Carboxylase (ACC) Activity Assay Kit can detect animal and plant tissues, bacteria and biological samples such as cells, serum or plasma. In this kit, ACC was able to catalyze acetyl-coa, NaHCO3 and ATP to produce malonyl-CoA, ATP and inorganic phosphorus, and ACC activity was determined by measuring the increase in inorganic phosphorus by ammonium molybdate method.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃, protected from light
Reagent Ⅰ	5 mL	10 mL	4℃
Reagent Ⅱ	1	1	4℃, protected from light
Reagent Ⅲ	1	1	-20℃, protected from light
Reagent Ⅳ	1	1	4℃, protected from light
Reagent Ⅴ	1	1	4℃, protected from light
Reagent Ⅵ	14.4 mL	28.8 mL	RT
Standard	10 mL	10 mL	4℃

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 660 nm

- 96-well microplate or microquartz cuvette, precision pipettes, disposable pipette tips
- Water bath, cryogenic centrifuge,1.5 mL EP tube
- Deionized water
- Mortar or homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Reagent II: Prepared before use. Add 1.25 mL Reagent Ⅰ to 48 T and 2.5 mL Reagent Ⅰ to 96 T, dissolve thoroughly. Store at 4℃, protected from light for 6 months.
Reagent III: Prepared before use. Add 1 mL deionized water to 48 T and 2 mL deionized wate to 96 T, dissolve thoroughly and set aside for use; Unused reagents can be stored at -20℃ for 6 months to avoid repeated freeze-thaw cycles.
Reagent IV: Prepared before use. Add 1.25 mL Reagent Ⅰ to 48 T and 2.5 mL Reagent Ⅰ to 96 T, dissolve thoroughly. Store at 4℃, protected from light for a week.
Reagent V: Prepared before use. Add 1.25 mL Reagent Ⅰ to 48 T and 2.5 mL Reagent Ⅰ to 96 T, dissolve thoroughly. Store at 4℃, protected from light for a week.
Reagent VI: Ready to use as supplied. Store at room temperature.
Working Reagent: According to the ratio of deionized water: Reagent Ⅳ: Reagent Ⅴ: Reagent Ⅵ=2: 1: 1: 1. Working Reagent is freshly prepared.
Standard： Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
0.5 μmol/mL Standard： Prepared before use. Add 0.5 mL standard into 9.5 mL deionized water, dissolve thoroughly.
Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma ,or other Liquid samples: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 660 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Preheat Reagent Ⅰ, Ⅱ and Ⅲ at 37℃ (mammals) or 25℃ (other species) for 10 min.
3. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Sample	0	0	10	10

Reagent Ⅰ	0	0	0	90
Reagent Ⅱ	0	0	50	0
Reagent Ⅲ	0	0	40	0
After an accurate reaction at 37℃ (mammals) or 25℃ (other species) for 30 min, the reaction was inactivated at 90℃ for 5 min (with a tight lid to prevent water loss), cooled, centrifuged at 10,000 g at 25℃ for 5 min, and the supernatant was removed. The following operations were operated in the 96-well microplate or microquartz cuvette:				
Supernatant	0	0	20	20
0.5 μmol/mL Standard	0	20	0	0
Deionized Water	20	0	0	0
Working Reagent	180	180	180	180

4. Mix thoroughly, after an accurate reaction at 37℃ (mammals) or 25℃ (other species) for 30 min, cool down to room temperature and measure the absorbance value at 660 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}, and the Control Well is marked as $A_{control}$. Finally calculate $\Delta A_{Test}=A_{Test}-A_{control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Each test well needs to be equipped with a control well, standard curve and blank well only need to be done once or twice. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.04, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.5, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Calculation of ACC activity:

(1) Calculated by protein concentration

Active unit definition: The production of 1 μmol of inorganic phosphorus produced per milligram of protein per hour was defined as one unit of enzyme activity.

$ACC(U/mg\ prot)=(C_{Standard}\times V_{Total})\times\Delta A_{Test}\div\Delta A_{Standard}\div(V_{Sample}\times Cpr)\div T=\mathbf{10\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr}$

(2) Calculated by fresh weight of samples

Active unit definition:The production of 1 μmol of inorganic phosphorus produced per gram tissue per hour was defined as one unit of enzyme activity.

$ACC(U/g\ fresh\ weight)=(C_{Standard}\times V_{Total})\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total}\div(W\times V_{Sample}\div V_{Total\ sample})\div T=\mathbf{10\times\Delta A_{Test}\div\Delta A_{Standard}\div W}$

(3) Calculated by bacteria or cells

Active unit definition:The production of 1 μmol of inorganic phosphorus produced per 10^4 bacteria or cells per hour was defined as one unit of enzyme activity.

$ACC(U/10^4)=(C_{Standard}\times V_{Total})\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total}\div(n\times V_{Sample}\div V_{Total\ sample})\div T=\mathbf{10\times\Delta A_{Test}\div\Delta A_{Standard}\div n}$

(4) Calculated by volume of liquid samples

Active unit definition:The production of 1 μmol of inorganic phosphorus produced per milliliter of liquid samples per hour was defined as one unit of enzyme activity.

$ACC(U/mL)=(C_{Standard}\times V_{Total})\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total}\div(V_{Sample}\div V_{Total\ sample})\div T=\mathbf{10\times\Delta A_{Test}\div\Delta A_{Standard}}$

$C_{Standard}$: the concentration of the standard, 0.5 μmol/mL; V_{Total}: total reaction volume, 0.1 mL; $V_{Sample:}$ sample volume added, 0.01 mL; $V_{Sample\ tota}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; T: reaction time, 30 min. n: The number of bacteria or cells, in tens of thousands; W: weight of sample, g.

Precautions

1. It is best to use new beakers, glass rods and glass pipettes to prepare Working reagents. Disposable plastic utensils can also be used to avoid phosphorus pollution.
2. The prepared Working Reagent should be light yellow, if it is colorless, the reagent will fail, if it is blue, it will be phosphorus pollution.
3. After the end of the color should be immediately tested, it is best to do this experiment at the same time, a person color, a person time, to ensure the accuracy of the experimental results.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

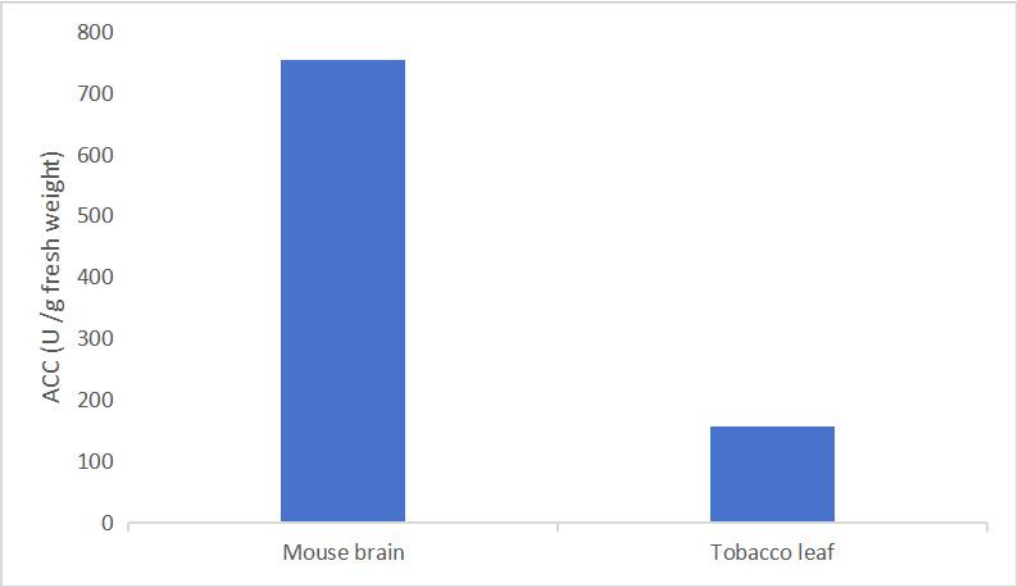

Figure 1. Determination ACC activity in mouse brain and tobacco leaf by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB3030	CheKine™ Micro Alcohol Dehydrogenase (ADH) Activity Assay Kit
KTB1560	CheKine™ Micro Alcohol Acyltransferase (AAT) Activity Assay Kit
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.