（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K508-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（650-670 nm）

Detection range：6.78-138 U/L

Elabscience® Acetyl-CoA Carboxylase(ACC) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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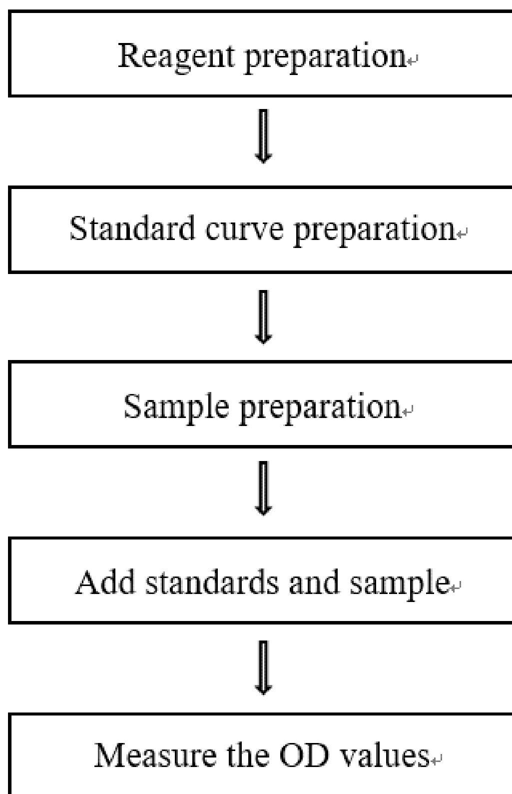
Assay summary



Intended use

This kit can be used to measure acetyl-coA carboxylase (ACC) activity in animal and plant tissue samples.

Detection principle

Acetyl-coA carboxylase (ACC) is a key enzyme in fatty acid anabolism, It catalyzes acetyl-coA to malonyl-coA, which is the donor of two carbon units in fatty acid synthesis. Therefore, ACC has become the rate-limiting enzyme in fatty acid anabolism, and is widely used in the research of transgenic oil crops.
ACC catalyzes acetyl-coA, $NaHCO_3$ and ATP to produce inorganic phosphorus, the activity of ACC can be calculated by measuring the change of inorganic phosphorus content.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	30 mL ×1 vial	-20 ℃, 12 months
Reagent 2	Substrate A	Powder ×2 vials	-20 ℃, 12 months shading light
Reagent 3	Negative Control Solution	3.5 mL ×1 vial	-20 ℃, 12 months
Reagent 4	Substrate B	3.5 mL ×1 vial	-20 ℃, 12 months shading light
Reagent 5	Chromogenic Agent A	Powder ×2 vials	-20 ℃, 12 months shading light
Reagent 6	Chromogenic Agent B	Powder ×2 vials	-20 ℃, 12 months shading light
Reagent 7	Acid Solution	1.8 mL ×1 vial	-20 ℃, 12 months
Reagent 8	500 μmol/L Standard Solution	6 mL ×1 vial	-20 ℃, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions

in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (650-670 nm, optimum wavelength: 660 nm), Vortex mixer, Incubator, Centrifuge, 100 ℃ water bath

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate A working solution:
Dissolve a vial of substrate A with 5 mL double distilled water. Aliquoted storage at -20 ℃ for 7 days protected from light.

③ The preparation of chromogenic agent A working solution:
Dissolve a vial of chromogenic agent A with 1 mL double distilled water. Store at 2-8 ℃ for 7 days protected from light.

④ The preparation of chromogenic agent B working solution:
Dissolve a vial of chromogenic agent B with 1 mL double distilled water. Store at 2-8 ℃ for 7 days protected from light. To re-dissolve place in a water bath (90-100 ℃) and until the clarificant looks clear.

⑤ The preparation of phosphorus assay reagent:
For each well, prepare 50 μL of phosphorus assay reagent (mix well 20 μL of double distilled water, 10 μL of chromogenic agent A working solution, 10 μL of chromogenic agent B working solution and 10 μL of acid solution). Prepared solution should be pale yellow. Otherwise, it should be invalid or

phosphorus pollution The phosphorus assay reagent should be prepared on spot protected from light.

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 500 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 100, 150, 200, 300, 400, 450, 500 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**100**	**150**	**200**	**300**	**400**	**450**	**500**
500 μmol/L Standard Solution (μL)	0	40	60	80	120	160	180	200
Double distilled water (μL)	200	160	140	120	80	40	20	0

Sample preparation

① **Sample preparation**

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4 ℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Epipremnum aureum tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Potato tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① With the preparation of phosphorus assay reagent, glass container can be selected for preparation. After the glass container is repeatedly scrubbed before use, it is repeatedly rinsed 10 times with double distilled water. Prepared solution should be pale yellow. If it is green or blue, it should be invalid or phosphorus pollution and it needs to be re-prepared.

② During the operation, take supernatant for determination carefully, and do not take precipitate.

③ To avoid external phosphorus contamination, be careful during the experiment.

Operating steps

Enzymatic reaction

① Control tube: Take 200 μL of buffer solution to the 1.5 mL EP tube.

Sample tube: Take 200 μL of buffer solution to the 1.5 mL EP tube.

② Add 50 μL of substrate A working solution to each tube.

③ Add 40 μL of negative control solution to control tube; Add 40 μL of substrate B to sample tube.

④ Add 40 μL of sample to the control tube and sample tube. Mix fully and incubate at 37 ℃ for 30 min.

⑤ 95 ℃ water bath for 5 min, then centrifuge at 8000 g for 5 min and take the supernatant for detection.

Chromogenic reaction

① Standard well: Take 80 μL of standards with different concentrations to the corresponding wells.

Control well: Take 80 μL of supernatant of control tube to the corresponding wells.

Sample well: Take 80 μL the supernatant of sample tube to the corresponding wells.

② Add 50 μL of phosphorus assay reagent to each well.

③ Mix fully with microplate reader and incubate at 37 ℃ with shading light for 10 min. Measure the OD value of each well at 660 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Definition: The amount of ACC in 1 g tissue protein per 1 min that hydrolysis of substrate to produce 1 μmol inorganic phosphorus at 37°C is defined as 1 unit.

$$\text{ACC activity (U/gprot)} = (\Delta A_{660} - b) \div a \div T \times (V_1 \div V_2) \div C_{pr} \times f$$

[Note]

ΔA_{660}: ($OD_{Sample} - OD_{Control}$).

T: The time of enzymatic reaction, 30min.

V_1: The volume of enzymatic reaction, 0.33 mL.

V_2: The volume of sample in enzymatic reaction, 0.04 mL.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	12.80	56.50	103.00
%CV	4.2	4.0	3.8

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	12.80	56.50	103.00
%CV	7.4	8.2	8.4

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	126	265	438
Observed Conc. (μmol/L)	127.3	251.8	429.2
Recovery rate (%)	101	95	98

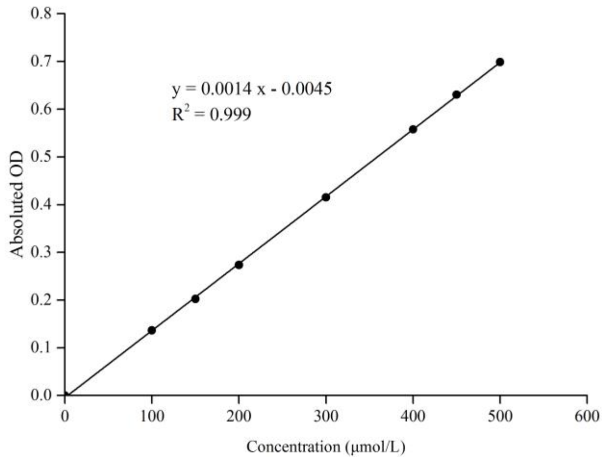
Sensitivity

The analytical sensitivity of the assay is 6.78 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**100**	**150**	**200**	**300**	**400**	**450**	**500**
OD value	0.040	0.181	0.243	0.315	0.452	0.596	0.670	0.733
	0.040	0.172	0.242	0.312	0.458	0.599	0.671	0.744
Average OD	0.040	0.177	0.243	0.314	0.455	0.598	0.671	0.739
Absoluted OD	0.000	0.137	0.203	0.274	0.415	0.558	0.631	0.699



Appendix Ⅱ Example Analysis

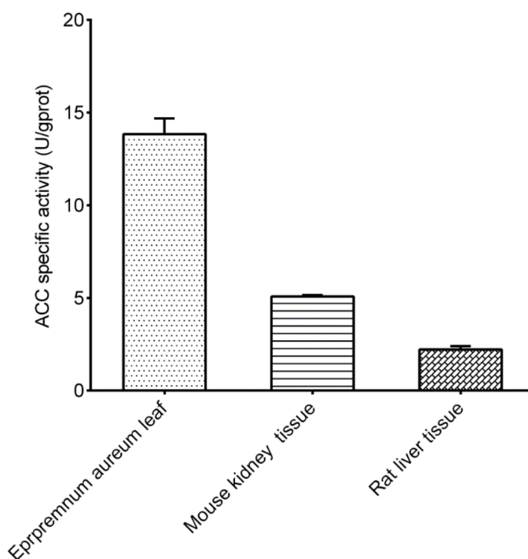
Example analysis:

For 10% Epipremnum aureum tissue homogenate, take 40 μL of sample and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0014 x - 0.0045, the average OD value of the control is 0.099, the average OD value of the sample is 0.188, the concentration of protein in sample is 1.26 gprot/L, and the calculation result is:

ACC activity (U/gprot) = (0.188 - 0.099 + 0.0045) ÷ 0.0014 ÷ 30 × (0.33 ÷ 0.04) ÷ 1.26 = 14.57 U/gprot

Detect 10% Epipremnum aureum tissue homogenate (the concentration of protein is 1.26 gprot/L), 10% Mouse kidney tissue homogenate (the concentration of protein is 6.98 gprot/L), 10% Rat liver tissue homogenate (the concentration of protein is 6.68 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.