TECHNICAL MANUAL

Acetyl-CoA Carboxylase (ACC) Activity Assay Kit

- **SKU CODE:** MAES0261
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Enzymatic reaction
- Chromogenic reaction
- Measurement and calculation

2. Intended use

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

3. 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, NaHCO3 and ATP to produce inorganic phosphorus. The activity of ACC can be calculated by measuring the change of inorganic phosphorus content.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	30 mL × 1 vial	-20°C, 12 months
Reagent 2	Substrate A	Powder × 2 vials	-20°C, 12 months, protect from light
Reagent 3	Negative Control Solution	3.5 mL × 1 vial	-20°C, 12 months
Reagent 4	Substrate B	3.5 mL × 1 vial	-20°C, 12 months, protect from light

Item	Component	Size (96 T)	Storage
Reagent 5	Chromogenic Agent A	Powder × 2 vials	-20°C, 12 months, protect from light
Reagent 6	Chromogenic Agent B	Powder × 2 vials	-20°C, 12 months, protect from light
Reagent 7	Acid Solution	1.8 mL × 1 vial	-20°C, 12 months
Reagent 8	500 μmol/L Standard Solution	6 mL × 1 vial	-20°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate A working solution:** Dissolve one vial of substrate A with 5 mL double distilled water. Store aliquots at -20°C for 7 days, protected from light.
3. **Preparation of chromogenic agent A working solution:** Dissolve one vial of chromogenic agent A with 1 mL double distilled water. Store at 2-8°C for 7 days, protected from light.
4. **Preparation of chromogenic agent B working solution:** Dissolve one vial of chromogenic agent B with 1 mL double distilled water. Store at 2-8°C for 7 days, protected from light. To redissolve, place in a water bath (90-100°C) until the solution appears clear.
5. **Preparation of phosphorus assay reagent:** For each well, prepare 50 μL of phosphorus assay reagent by mixing 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. The prepared solution should be pale yellow. If it appears green or blue, it is invalid or contaminated with phosphorus and should be reprepared. The phosphorus assay reagent should be prepared immediately before use and protected from light.

6. **Preparation of standard curve:** Always prepare fresh standards. Discard working standard dilutions after use. Dilute 500 µmol/L standard solution with double distilled water to serial concentrations. The recommended dilution gradient is: 0, 100, 150, 200, 300, 400, 450, 500 µmol/L.

7. Sample preparation

Tissue sample preparation:

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°C.

4. Centrifuge at 10,000×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 (MAES0177).

Dilution of sample:

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

- 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 perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. For preparation of phosphorus assay reagent, select a glass container. After repeatedly scrubbing the glass container before use, rinse it 10 times with double distilled water. The prepared solution should be pale yellow. If it is green or blue, it is invalid or contaminated with phosphorus and needs to be reprepared.

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

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

9. Operating steps

Enzymatic reaction:

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

Sample tube: Take 200 μL of buffer solution to a 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 both the control tube and sample tube. Mix thoroughly and incubate at 37°C for 30 min.

5. Place in 95°C water bath for 5 min, then centrifuge at 8,000×g for 5 min and take the supernatant for detection.

Chromogenic reaction:

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

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

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

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

3. Mix thoroughly with microplate reader and incubate at 37°C, protected from light, for 10 min. Measure the OD value of each well at 660 nm with microplate reader.

10. 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 absolute OD value.

3. Plot the standard curve using absolute OD value of standards and corresponding 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 hydrolyzes substrate to produce 1 µmol inorganic phosphorus at 37°C is defined as 1 unit.

ACC activity (U/g prot) = (ΔA_660 - b) ÷ a ÷ T × (V_1 ÷ V_2) ÷ C_{pr} × f

[Note]

ΔA_660: (OD_{Sample} – $OD_{Control}$)

T: The time of enzymatic reaction, 30 min

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, g prot/L

f: Dilution factor of sample before test

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

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

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with 6 parallel tests for each concentration to obtain an average recovery rate of 98%.

Recovery

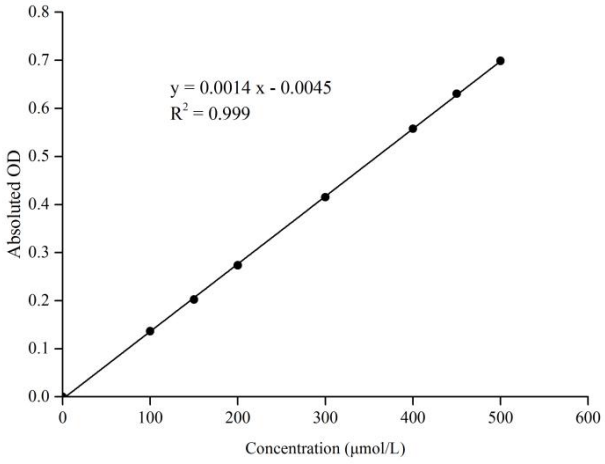
	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	126	265	438
Observed Conc. (µmol/L)	127.3	251.8	429.2
Recovery rate (%)	101	95	98

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.

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), the standard curve and data are provided below for reference only:



Standard curve

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

12. Appendix II Example Analysis

Example analysis:

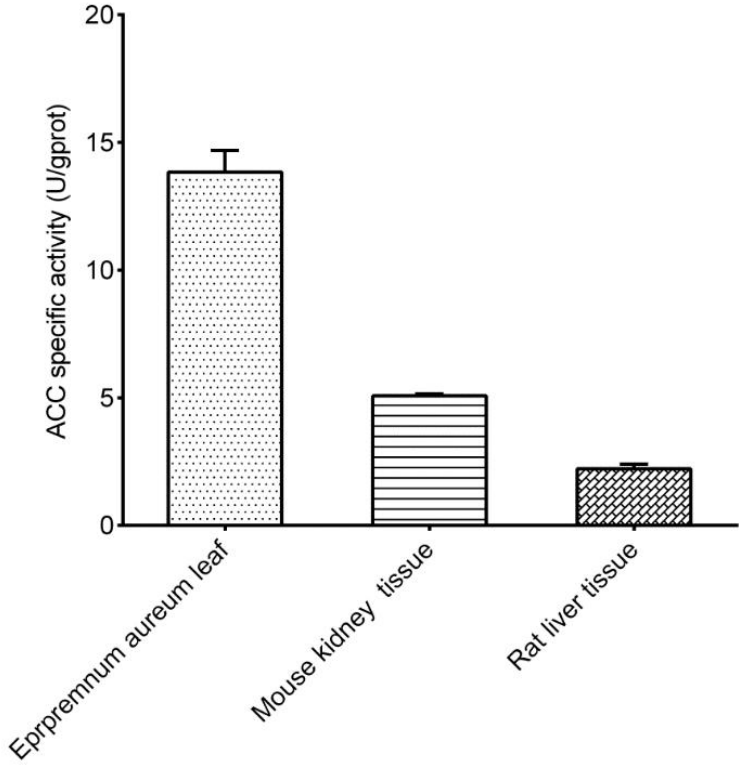
For 10% Epipremnum aureum tissue homogenate, take 40 μL of sample and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0014x - 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 g prot/L, and the calculation result is:

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

= 14.57 U/g prot

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



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.