PRODUCT INFORMATION & MANUAL

Acetyl-CoA Carboxylase Activity Assay Kit (Colorimetric)

NBP3-24454

For research use only.
Not for diagnostic or therapeutic procedures.

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Acetyl-CoA Carboxylase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24454

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 6.78 U/L

Detection range: 6.78-138 U/L

Average intra-assay CV (%): 4.0

Average inter-assay CV (%): 8.0

Average recovery rate (%): 98

▲ This kit is for research use only.
▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲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	Specification	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.			

▲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)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ 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.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 2 working solution:
   Dissolve a vial of reagent 2 with 5 mL double distilled water. The prepared solution can be divided into smaller packages at -20°C with shading light for 7 days.
3. Preparation of reagent 5 working solution:
   Dissolve a vial of reagent 5 with 1 mL double distilled water. The prepared solution can be stored at 2-8°C with shading light for 7 days.
4. Preparation of reagent 6 working solution:
   Dissolve a vial of reagent 6 with 1 mL double distilled water. The prepared solution can be stored at 2-8°C with shading light for 7 days. If any solid is insoluble, it can be dissolved in 90-100°C water bath..
5. Preparation of phosphorus assay reagent:
   Mix the double distilled water, reagent 5 working solution, reagent 6 working solution and reagent 7 at the ratio of 2:1:1:1 fully. Prepared solution should be pale yellow. Otherwise, it should be invalid or phosphorus pollution. Prepare the fresh phosphorus assay reagent before use and the prepared solution should be stored with shading light

▲ Sample preparation

Tissue sample:

Accurately weigh the tissue, add normal saline (0.9% NaCl) at a ratio of Weight (g): Volume (mL) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 10 min at 4 °C, and take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range 6.78-138 U/L).

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).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note:A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

▲Detailed operation steps

1. The preparation of standard

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:

Number	Standard concentrations (μmol/L)	500 μmol/L standard solution (μL)	double distilled water (μL)
A	0	0	200
B	100	40	160
C	150	60	140
D	200	80	120
E	300	120	80
F	400	160	40
G	450	180	20
H	500	200	0

2. The measurement of samples

2.1 Enzymatic reaction

(1) Control tube: Take 200 μL of reagent 1 to the 1.5 mL EP tube.

Sample tube: Take 200 μL of reagent 1 to the 1.5 mL EP tube.

(2) Add 50 μL of reagent 2 working solution to each tube.

(3) Add 40 μL of regent 3 to control tube; Add 40 μL of regent 4 to sample tube.

(4) Add 40 μL of sample to the control tube and sample tube. Mix fully and incubate at 37°Cfor 30 min

(5) 95°C water bath for 5 min, then centrifuge at 8000 g for 5 min and take the supernatant for detection.

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

▲Summary operation table

1. Enzymatic reaction

	Sample tube	Control tube
Regent 1 (μL)	200	200
Regent 2 working solution (μL)	50	50
Regent 3 (μL)	--	40
Regent 4 (μL)	40	--
Sample (μL)	40	40
Mix fully and incubate at 37°C for 30 min.		
95°Cwater bath for 5 min, then centrifuge at 8000 g for 5 min and take the supernatant for detection.		

2. Chromogenic reaction

	Standard well	Sample well	Control well
Standard solution with different concentrations (μL)	80		
Supernatant of sample tube (μL)		80	
Supernatant of control tube (μL)			80
Phosphorus assay reagent (μL)	50	50	50
Mix fully with microplate reader and incubate at 37°C with shading light for 10 min. Measure the OD value of each well at 660 nm with microplate reader.			

Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

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:

y: $OD_{Standard} - OD_{Blank}$, ($OD_{Blank}$ is the OD value when the standard concentration is 0).

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve.

ΔA660: ($OD_{Sample} - OD_{Control}$).

T: The time of enzymatic reaction, 30min.

V1: The volume of enzymatic reaction, 0.33 mL.

V2: 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 Data

▲Example analysis

For 10% Epipremnum aureum tissue homogenate, take 40 μL of sample and carry the assay according to the operation table.

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