Acetyl-CoA Carboxylase

Microplate Assay Kit

Cat #: orb545618 (manual)

Detection and Quantification of Acetyl-CoA Carboxylase activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

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INTRODUCTION

Acetyl-CoA carboxylase (ACC) is a biotin-dependent enzyme that catalyzes the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA through its two catalytic activities, biotin carboxylase (BC) and carboxyltransferase (CT). ACC is a multi-subunit enzyme in most prokaryotes and in the chloroplasts of most plants and algae, whereas it is a large, multi-domain enzyme in the endoplasmic reticulum of most eukaryotes. The most important function of ACC is to provide the malonyl-CoA substrate for the biosynthesis of fatty acids. The activity of ACC can be controlled at the transcriptional level as well as by small molecule modulators and covalent modification.

Acetyl-CoA carboxylase can react with acetyl-CoA and ATP, the products are malonyl-CoA, ADP and inorganic phosphorus. The Acetyl-CoA carboxylase activity will be determined by the increase of inorganic phosphorus. At the end of the reaction period, the dye reagent forms a color with released phosphate, which is measured on a plate reader 635 nm.

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	8 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Dye Reagent I	6 ml x 1	4 °C
Dye Reagent II	Powder x 1	4 °C
Dye Reagent II Diluent	9 ml x 1	4 °C
Standard (100 µmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 8 ml Reaction Buffer before use.

Dye Reagent II: add 9 ml Dye Reagent II Diluent and heat to dissolve before use, store at 4 °C.

Dye Reagent Working Solution: add 9 ml Dye Reagent II into 6 ml Dye Reagent I, mix, store at 4 °C for 2-3 days.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 635 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times) ; centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank
Sample	20 µl	--	--	--
Assay Buffer	--	20 µl	--	--
Substrate	80 µl	80 µl	--	--
Mix, put it in the oven, 37 °C for 30 minutes. Then put it in boiling water for 5 minutes. When cold, centrifuged at 10000g, room temperature for 5 minutes. Add following reagents into the 96-Well microplate.				
Standard	--	--	50 µl	--
Distilled water	--	--	--	50 µl
Supernatant	50 µl	50 µl	--	--
Dye Reagent Working Solution	150 µl	150 µl	150 µl	150 µl
Mix, room temperature for 5 minutes, record absorbance measured at 635nm.				

Note:

1) It is best to use disposable plastic tube to avoid phosphorus pollution.

2) Perform 2-fold serial dilutions of the top standards to make the standard curve.

3) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

CALCULATION

Unit Definition: One unit of Acetyl-CoA Carboxylase activity is defined as the enzyme generates 1 nmol of PO_4^{3-} per hour.

1. According to the protein concentration of sample

$$ACC\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T \times 2$$

$$= 400 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$ACC\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T \times 2$$

$$= 400 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

$C_{Standard}$: the concentration of Standard, 100 μmol/L = 100 nmol/ml;

$V_{Standard}$: the volume of the Standard, 0.05 ml;

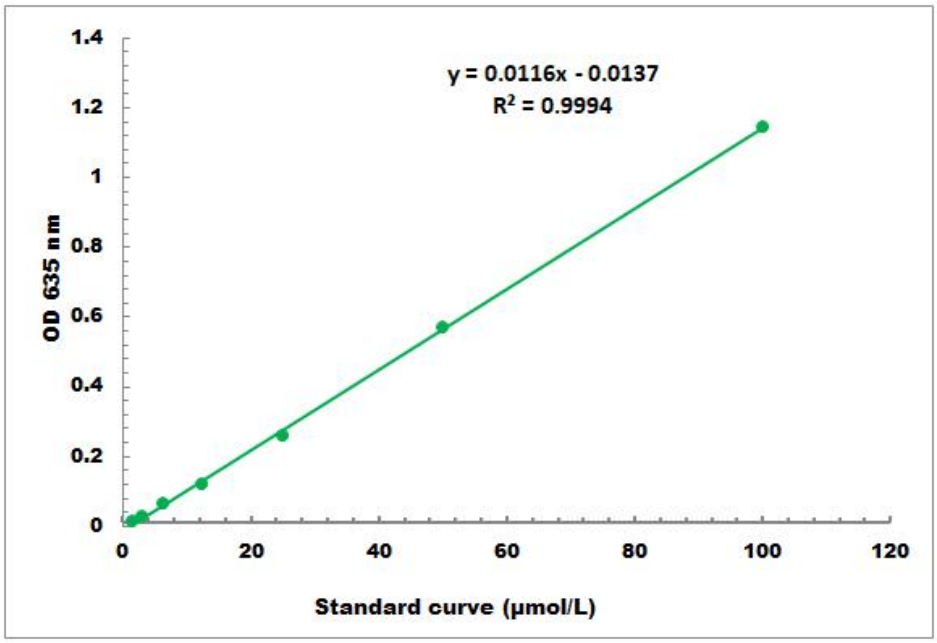
V_{Sample}: the volume of sample, 0.05 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 0.5 h.

TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 1 µmol/L - 100 µmol/L

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