Acetyl-CoA Carboxylase Activity ColorimetricMicroplate Assay Kit User Manual

Catalog # CAK1138

(Version 1.4C)

Detection and Quantification of Acetyl-CoA Carboxylaseactivity inTissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. 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 ActivityColorimetric Microplate Assay Kit provides a sensitive and convenient method for acetyl-CoA carboxylase activity determination.

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.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 4	4 °C
Assay Buffer II	Powder x 1	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 (100μmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. 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

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 100/50/25/12.5/6.25/3.12/1.56μmol/L.

Assay Buffer II: Briefly centrifuge prior to opening. Add 1.2 ml ethanol to dissolve before use. Keep in dark for immediate use or store at -20°C for 3-4 months after reconstitution.

Substrate: Add 8 mlReaction Bufferbefore use.Keep in dark for immediate use or store at -20°C for 2-3 weeks after reconstitution.

Dye Reagent II: Add 9 mlDye Reagent II Diluent and heat to dissolve before use,store at 4 °C.Keep in dark and store at 4 °C for 2-3 months after reconstitution.

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.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.99 mlAssay Buffer Iand 10 µl Assay Buffer II for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10minutes, 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 0.99 mlAssay Buffer Iand 10 µl Assay Buffer II on ice, centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Add following reagents intothe 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 intothe 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 temperaturefor 5 minutes, record absorbance measured at 635nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of Acetyl-CoA Carboxylaseactivityis defined asthe enzyme generates 1μmol of PO_4^{3-}per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2 According to the weight of sample

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

2.3 According to the volume of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

$C_{Standard}$: the concentration of standard, μmol/L

$V_{Standard}$: the volume of standard in assay procedure, mL

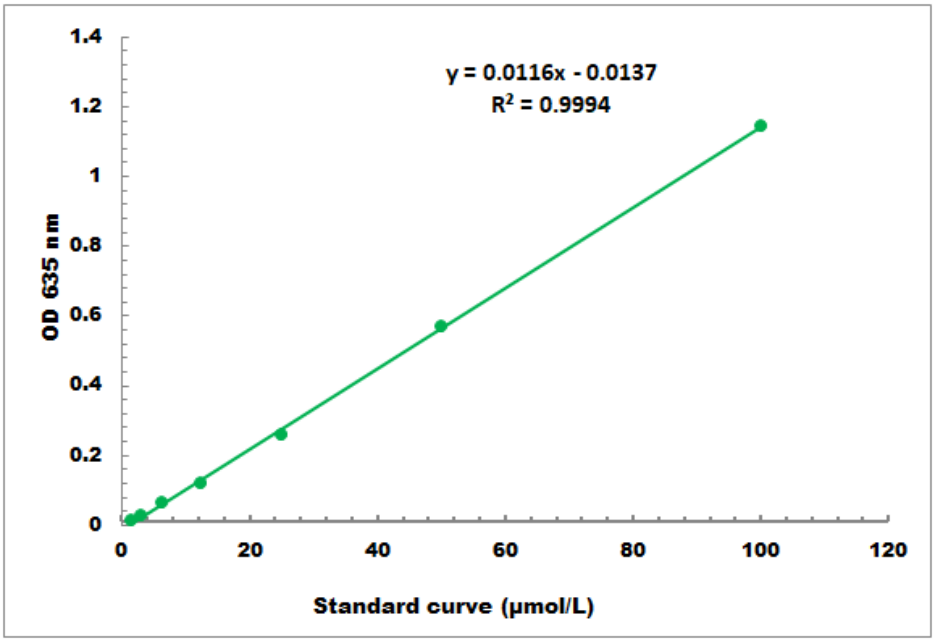
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 1 μmol/L - 100μmol/L