Acetyl Coenzyme A

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1144

(Version 1.2B)

Detection and Quantification of Acetyl Coenzyme A Content in Tissue extracts, Cell lysate Samples.

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

I. INTRODUCTION

Acetyl Coenzyme A (Acetyl-CoA) serves as the central hub linking carbohydrate, lipid, and protein metabolism, feeding acetyl groups into the TCA cycle for energy production. It also acts as a key precursor for synthesizing fatty acids, cholesterol, and neurotransmitters. Measuring its levels aids in assessing mitochondrial function and metabolic disorders.

Acetyl-CoA Colorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of Acetyl-CoA in a variety of samples. In this assay, an acetyl group is transferred from CoA to Carnitine and the free CoA, which reacts with probe to form a colorimetric (412 nm) product, proportional to Acetyl-CoA.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	16 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 2	4 °C, keep in dark
Dye Reagent Diluent	2 ml x 1	4 °C
Standard Diluent	2 ml x 1	4 °C
Standard	Powder x 1	4 °C, keep in dark
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 412 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 0.1 ml Standard Diluent to generate 10 mmol/L of top standard solution. Keep in dark and store at 4°C for 12 hours or -20 °C for 1 week. Then perform 2-fold serial dilutions of the top standard solution using Standard Diluent to make the standard curve. The concentration of standard curve could be 10/5/2.5/1.25/0.625/ 0.312/0.156 mmol/L.

Substrate: Briefly centrifuge prior to opening. Dissolve each substrate in 1.5 ml distilled water before use. Store at 4 °C for 2 weeks.

Enzyme: Briefly centrifuge prior to opening. Dissolve in 0.5 ml Reaction Buffer and 0.5 ml distilled water mixture before use. Store at -80 °C for 1 month.

Dye Reagent: Briefly centrifuge prior to opening. Dissolve each vial in 1 ml Dye Reagent Diluent before use. Keep in dark and store at -20 °C for 1 month or 4°C for 7 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 1 ml Assay Buffer for 5×10^6 cell or bacteria (the quantity should be adjusted according to the actual situation of the sample), sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g 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 (the quantity should be adjusted according to the actual situation of the sample), homogenize with 1 ml Assay Buffer on ice, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Blank	Standard
Reaction Buffer	150 µl	150 µl	150 µl
Sample	10 µl	--	--
Distilled water	--	10 µl	--
Standard	--	--	10 µl
Enzyme	10 µl	10 µl	10 µl
Substrate	15 µl	15 µl	15 µl
Dye Reagent	20 µl	20 µl	20 µl
Mix and incubate at 37 °C for 10 minutes, record absorbance measured at 412 nm			

Note:

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

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**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

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \ (mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

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

2.1 According to the quantity of cells or bacteria

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay})} \ (\mu mol/10^4)$$

2.2 According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay})} \ (\mu mol/g)$$

2.3 According to the liquid of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \ (\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

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

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

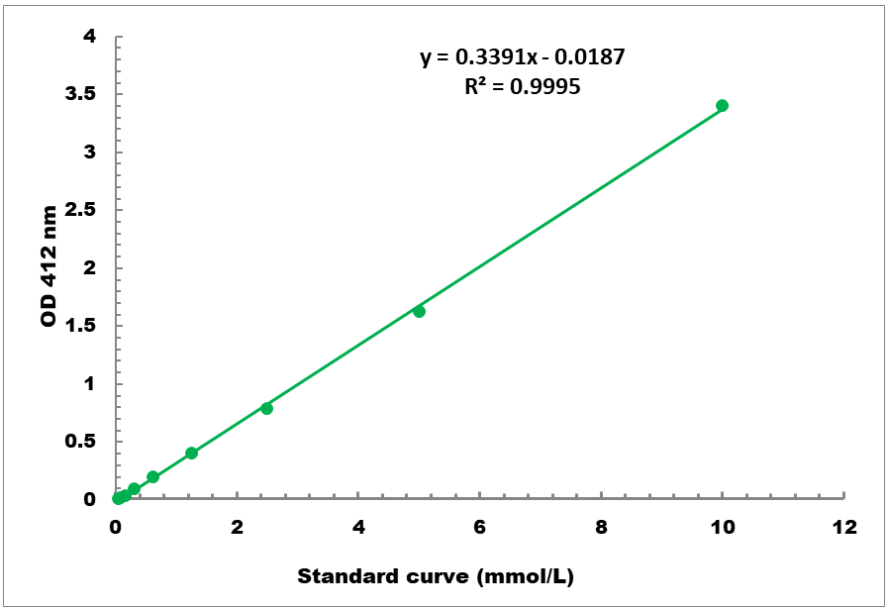
V_{Assay}: the volume of Assay Buffer, μl

W: the weight of sample, g

N: the quantity of cell or bacteria, 10^4

VIII. TYPICAL DATA

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



Detection Range: 0.1 mmol/L - 10 mmol/L