Carnitine Acetyltransferase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1324

(Version 1.1A)

Detection and Quantification of Carnitine Acetyltransferase (CRAT) Activity in Tissue extracts, Cell lysate Samples.

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

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I. INTRODUCTION

Carnitine acetyltransferase (CRAT) is a pivotal metabolic enzyme that catalyzes the transfer of acetyl groups from acetyl-CoA to L-carnitine. This reaction plays a central role in cellular energy metabolism by regulating the acetyl-CoA/CoA ratio in mitochondria and cytoplasm, thereby maintaining dynamic equilibrium of acetyl groups. Furthermore, CRAT participates in fatty acid oxidation, ketone body metabolism, antioxidant defense systems and neurodegenerative diseases.

Carnitine Acetyltransferase Activity Colorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of carnitine acetyltransferase activity in a variety of samples. In this assay, acetyl group is transferred from acetyl-CoA producing free CoA, which reacts with probe to form a colorimetric (450 nm) product, proportional to the CRAT activity present.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	25 ml x 1	4 °C
Coenzyme	Powder x 2	-20 °C, keep in dark
Substrate	Powder x 2	4 °C
Antioxidant Diluent	1 ml x 2	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Dye Reagent Diluent	2 ml x 1	4 °C
Standard	Powder x 1	4 °C, keep in dark
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml Antioxidant Diluent to generate 2 mmol/L of top standard solution. Then perform 2-fold serial dilutions of the top standard solution using Reaction Buffer to make the standard curve. The concentration of standard curve could be 2.0/1.0/0.5/0.25/0.125/0.0625/0.0312/0.0156/0.0078 mmol/L. Store at -20 °C for 1 weeks or 4°C for 3 days in dark.

Coenzyme: Briefly centrifuge prior to opening. Dissolve each Coenzyme vial in 0.5 ml Antioxidant Diluent. Prepare fresh for immediate use. Keep rest solution in dark and store at 4°C for 12 hours or -20 °C for 1 week.

Substrate: Briefly centrifuge prior to opening. Dissolve each substrate in 1 ml distilled water before use. Store at 4 °C for 2 weeks.

Positive Control: Briefly centrifuge prior to opening. Dissolve in 60 ul Assay Buffer before use. Store at -80 °C for 1 month.

Dye Reagent: Briefly centrifuge prior to opening. Dissolve in 2 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**	Control	Positive Control	Standard	Blank
Reaction Buffer	140 µl	140 µl	140 µl	140 µl	140 µl
Distilled water	--	10 µl	--	--	40 µl
Coenzyme	10 µl	10 µl	10 µl	--	--
Sample	10 µl	--	--	--	--
Positive control	--	--	10 µl	--	--
Substrate	20 µl	20 µl	20 µl	--	--
Standard	--	--	--	40 µl	--
Dye Reagent	20 µl	20 µl	20 µl	20 µl	20 µl
Mix and incubate at room temperature for 20 minutes, record absorbance measured at 450 nm.					

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.

VII. CALCULATION

Unit Definition: One unit of CRAT is defined as the enzyme catalyzes the transfer of acetyl groups and yielding 1 μmol of CoA per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T} \quad (U/10^4)$$

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

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

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

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

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

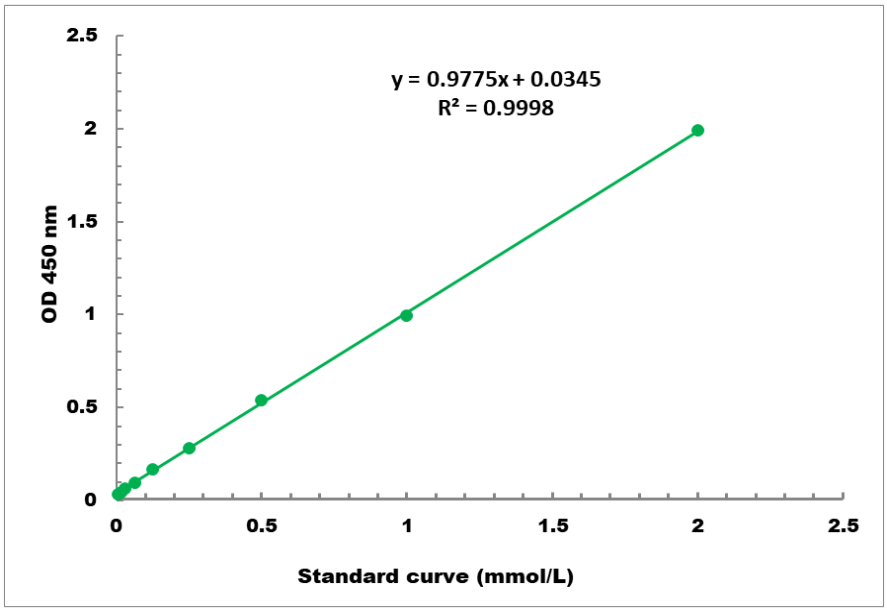
V_{Sample}: the volume of sample in assay procedure, mL

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

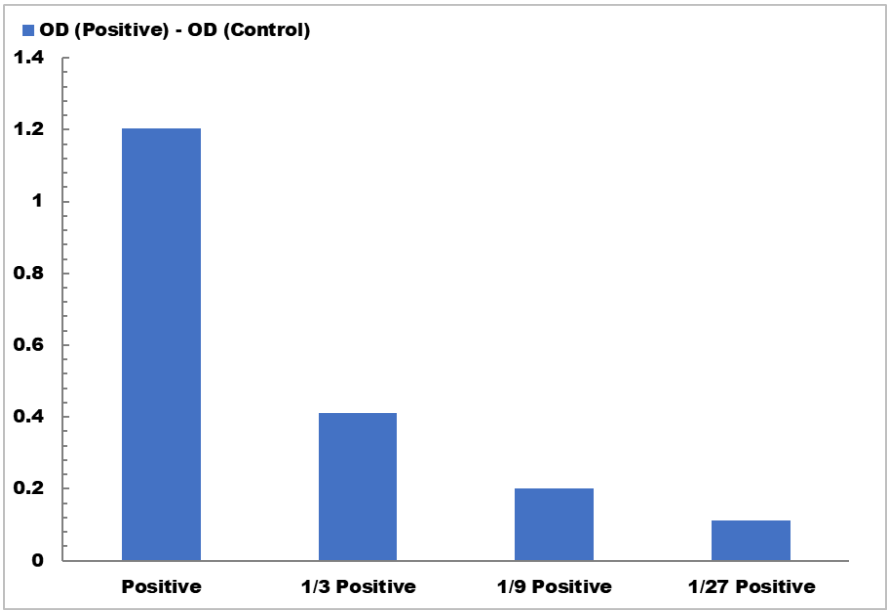
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 0.008 mmol/L - 2 mmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration