L-Carnitine

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1244

(Version 1.1A)

Detection and Quantification of L-Carnitine Content in Tissue extracts, Cell lysate Samples.

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

I. INTRODUCTION

Carnitine is a quaternary ammonium compound biosynthesized from lysine and methionine, which is critical in energy metabolism. As an essential carrier for fatty acid β-oxidation, it facilitates the transport of long-chain fatty acids into the mitochondrial matrix via acylcarnitine formation, thereby promoting ATP production and functioning as a critical nexus linking lipid metabolism with the tricarboxylic acid (TCA) cycle. Carnitine exists in two stereoisomers. Only L-carnitine is biologically active.

L-carnitine Colorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of L-carnitine 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 (450 nm) product, proportional to L-carnitine.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	13 ml x 1	4 °C
Coenzyme	Powder x 2	-20 °C, keep in dark
Coenzyme Diluent	1 ml x 1	4 °C
Enzyme	Powder x 1	-20 °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
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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. Convection oven

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml Assay Buffer to generate 10 mmol/L of top standard solution. Then perform 2-fold serial dilutions of the top standard solution using Assay Buffer to make the standard curve. The concentration of standard curve could be 10.0/5.0/2.5/1.25/0.625/0.312/0.156 mmol/L. Store at 4°C for 7 days.

Coenzyme: Briefly centrifuge prior to opening. Dissolve each Coenzyme vial in 0.5 ml Coenzyme 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.

Enzyme: Briefly centrifuge prior to opening. Dissolve in 2 ml 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**	Blank	Standard
Reaction Buffer	130 µl	130 µl	130 µl
Distilled water	--	20 µl	--
Coenzyme	10 µl	10 µl	10 µl
Enzyme	20 µl	20 µl	20 µl
Sample	20 µl	--	--
Standard	--	--	20 µl
Dye Reagent	20 µl	20 µl	20 µl
Mix and incubate at room temperature for 10 minutes, record absorbance measured at 450 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.

VII. CALCULATION

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

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

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 protein concentration of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times C_{Protein} \times V_{Sample}} \ (mg/mg)$$

2.2 According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mg/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

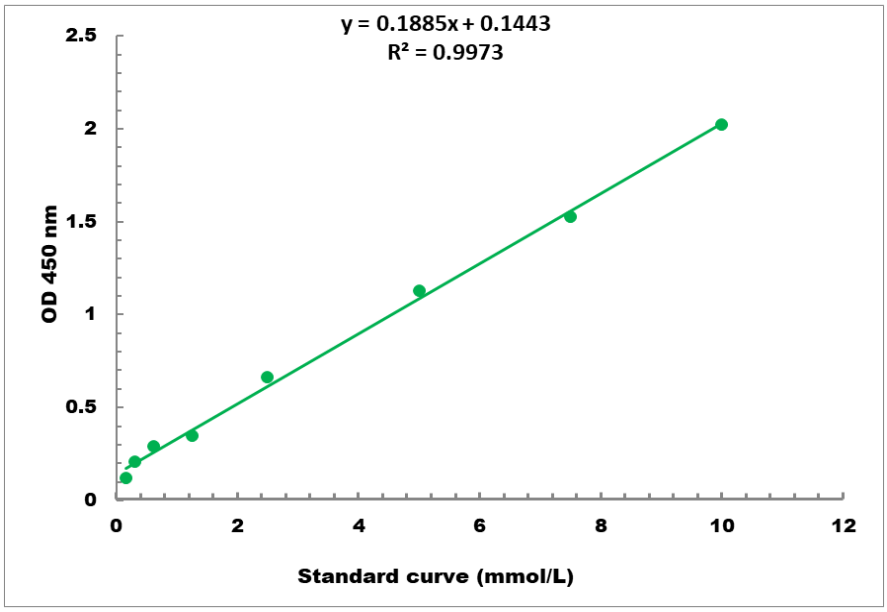
$C_{Protein}$: the sample protein concentration, mg/ml

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