EnzyChrom™ L-Carnitine Assay Kit (ELCR-100)

Quantitative Colorimetric/Fluorimetric L-Carnitine Determination

DESCRIPTION

L-CARNITINE is found in most mammals, plants, and some bacteria. L-Carnitine's most crucial role is in the transport of long-chain fatty acids from the cytosol into the mitochondria for fatty acid oxidation. BioAssay Systems' method provides a simple and high-throughput assay for measuring L-Carnitine. In this assay, a multistep reaction produces H_2O_2 which reacts with a specific dye to form a pink colored product. The optical density at 570nm or fluorescence intensity at $\lambda_{ex/em}$ = 530/585 nm is directly proportional to the L-Carnitine concentration in the sample.

KEY FEATURES

Sensitive. Uses 10 µL samples. Detection range: colorimetric assay 12 - 1000 µM, fluorimetric assay 1 - 100 µM L-Carnitine.

Convenient. Room temperature "mix-incubate-read" procedure can be readily automated for high-throughput assay of thousands of samples per day.

APPLICATIONS

Assays: L-Carnitine in urine, serum, plasma, cell lysates, tissue lysates and food products (e.g. milk).

KIT CONTENTS

Assay Buffer:	10 mL	**Dye Reagent:**	120 µL
Enzyme A:	Dried	**Enzyme B:**	100 µL
Enzyme C:	100 µL	**Standard**:	50 µL
Substrate Mix:	400 µL		

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Safety Data Sheet for detailed information.

PROCEDURES

Reagent Preparation:

Reconstitute Enzyme A by adding 120 µL dH_2O to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down and incubating at RT for 15 min. Store reconstituted Enzyme A at -20°C and use within 2 months.

Sample Preparation:

Serum, plasma, and milk should be clarified by perchloric acid:potassium hydroxide precipitation (see below). Serum and Plasma should be diluted no more than 1:5 with an internal standard. Cell lysates may be assayed directly. Milk can be measured directly or with an internal standard.

For perchloric acid:potassium hydroxide clarification, add 10 µL of 60% perchloric acid to 100 µL of sample, mix well, and centrifuge for 5 minutes at 14k rpm. Add 40 µL of 2 M KOH to 100 µL of supernatant, allow to precipitate on ice, and then centrifuge for 2 minutes at 14k rpm to collect the supernatant. The dilution factor *n* is 1.54.

Note: SH-containing reagents (e.g. β–mercaptoethanol, or dithiothreitol, > 5 µM), sodium azide, EDTA, and sodium dodecyl sulfate are known to interfere in this assay and should be avoided in sample preparation.

Fluorimetric Assay:

1. Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay. *Important: The Substrate Mix may be slightly turbid, but the turbidity does not interfere with the reaction.*
2. *Standards*: Prepare a 100 µM stock of standard by diluting 1 µL of the 100 mM Standard with 999 µL of dH_2O. Dilute the 100 µM standard in Assay Buffer as follows:

No	100 µM STD + dH_2O	Vol (µL)	L-Carnitine (µM)
1	100 µL + 0 µL	100	100
2	60 µL + 40 µL	100	60
3	30 µL + 70 µL	100	30
4	0 µL +100 µL	100	0

Transfer 10 µL of each standard into separate wells of a black 96-well plate.

Samples: Transfer 10 µL of each sample into separate wells of the plate.

Samples requiring an internal standard will need three separate reactions: 1) Sample plus Standard, 2) Sample alone and 3) Sample Blank. For the Sample and Sample Blank, add 10 µL of sample to the Sample and Sample Blank wells, respectively. For the Sample plus Standard well, add 1 µL of 1 mM Standard to 39 µL of sample, mix well, and transfer 10 µL to the well.

3. *Fluorimetric Reaction*. Prepare enough Working Reagent by mixing, for each well, 95 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B, 1 µL Enzyme C, 1 µL Dye Reagent and 4 µL Substrate Mix. Add 90 µL of WR to each well, tap to mix and incubate at room temperature (RT) for 30 min protected from light. If using an Internal Standard, prepare a Sample Blank Working Reagent with all the components for the Working Reagent except Enzyme A. Add 90 µL of Sample Blank Working Reagent to the Sample Blank wells.
4. Read fluorescence ($\lambda_{ex/em}$ = 530/585 nm) at immediately (t_{0min}) and at 30 min (t_{30min}), or record kinetics for 30 min.

Colorimetric Assay:

The colorimetric assay procedure is similar to the fluorimetric procedure except that (1) 0, 300, 600 and 1000 µM Standards and (2) a clear 96-well plate are used. Read optical density at 570 nm.

CALCULATION

For samples without background matrix effects, use values obtained at 30min. Subtract Blank value (Standard #4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the L-Carnitine concentration of Sample,

$$[\text{L-Carnitine}] = \frac{R_S - R_{BL}}{\text{Slope } (\mu M^{-1})} \times n\ (\mu M)$$

For samples with background matrix effects (e.g. milk and serum), an internal standard should be used, the sample L-Carnitine concentration is calculated as follows:

$$[\text{L-Carnitine}] = \frac{\Delta R_S - \Delta R_{SB}}{\Delta R_{IS} - \Delta R_S} \times n \times 25\ (\mu M)$$

Where ΔR_S, ΔR_{SB}, and ΔR_{IS} are the fluorescence or optical density readings of the Sample, Sample Blank and Sample plus Internal Standard, respectively, at $t_{30min} - t_{0min}$. ***n*** is the sample dilution factor. If the standard curve is non-linear, use polynomial fitting to solve for the concentration.

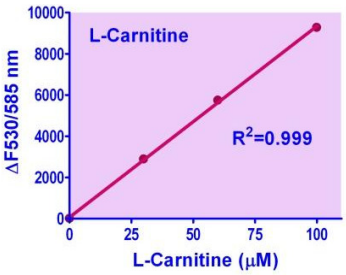
Note: If the calculated L-Carnitine concentration of a sample is higher than 1000 µM in the Colorimetric Assay or 100 µM in the Fluorimetric Assay, dilute sample in dH_2O and repeat the assay. Multiply result by the dilution factor, ***n***.

MATERIALS REQUIRED, BUT NOT PROVIDED

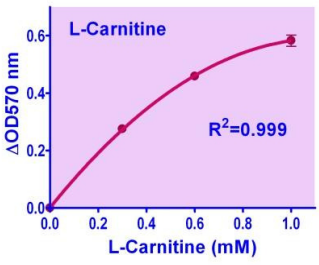
Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates (e.g. VWR cat# 82050-760), optical density plate reader, black flat-bottom uncoated 96-well plates (e.g. VWR cat# 82050-676), fluorescence plate reader. For milk, serum, and plasma samples: 60% perchloric acid and potassium hydroxide.

EXAMPLES

A PANC-1 cell lysate sample was assayed directly at 0.99 mg/mL protein concentration. The calculated L-Carnitine concentration was 23.2 +/-5.2 nmoles/mg total protein. Human serum and whole cow's milk were assayed using the perchloric acid:potassium hydroxide precipitation and internal standard method. The L-Carnitine concentrations were found to be 46.3 ± 5.9 µM (human serum) and 313.7 ± 85.1 µM (whole cow's milk), respectively.



96-well fluorimetric assay



96-well colorimetric assay

LITERATURE

1. Pekala J, et al. (2011). L-carnitine-metabolic functions and meaning in human life. Curr. Drug Metab. 12:667-78
2. Almannai M, et al. (1980). Carnitine inborn errors of metabolism. Molecules. 24: 3251-3267.
3. Longo N, et al. (2016). Carnitine transport and fatty acid oxidation. Biochim Biophys Acta. 1863:2422-35.