L-Carnitine Assay Kit

Product Datasheet | SKU BA0276

Description

L-Carnitine is found in most mammals, plants and some bacteria. Its most crucial role is in the transport of long-chain fatty acids from the cytosol into the mitochondria for fatty acid oxidation. This method provides a simple and high-throughput assay for measuring L-carnitine. In this assay, a multistep reaction produces H2O2 which reacts with a specific dye to form a pink coloured product. The optical density at 570 nm or fluorescence intensity at Ex/Em = 530/585 nm is directly proportional to the L-carnitine concentration in the sample.

Specifications

Detection Method	Colorimetric (OD 570 nm) or fluorimetric (Ex/Em = 530/585 nm)
Detection Range	Colorimetric assay: 12 - 1000 µM; fluorimetric assay: 1 - 100 µM L-Carnitine
Sample Type	Urine, serum, plasma, cell lysates, tissue lysates and food products (e.g. milk)
Species Reactivity	All
Assay Time	Approximately 30 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	The kit is shipped on ice. Store all components at -20°C.
Shelf Life	6 months after receipt
Shipping	Gel Pack

Kit Components

Component	Quantity	Storage
Assay Buffer	10 mL	-20°C
Dye Reagent	120 µL	-20°C
Enzyme A	Dried	-20°C
Enzyme B	100 µL	-20°C
Enzyme C	100 µL	-20°C
Standard	50 µL	-20°C
Substrate Mix	400 µL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0276	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Reconstitute Enzyme A by adding 120 µL dH2O to the Enzyme A tube, ensuring it is fully dissolved by pipetting up and down and incubating at room temperature for 15 min; store reconstituted Enzyme A at -20°C and use within 2 months.
2. Sample preparation: serum, plasma and milk should be clarified by perchloric acid:potassium hydroxide precipitation, with serum and plasma diluted no more than 1:5 with an internal standard; cell lysates may be assayed directly and milk can be measured directly or with an internal standard.

3. 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, then centrifuge for 2 minutes at 14k rpm to collect the supernatant (dilution factor n is 1.54).
4. Note: SH-containing reagents (e.g. beta-mercaptoethanol or dithiothreitol, > 5 µM), sodium azide, EDTA and sodium dodecyl sulfate interfere and should be avoided in sample preparation.
5. Equilibrate all components to room temperature, briefly centrifuge the tubes before opening and keep thawed tubes on ice during the assay; the Substrate Mix may be slightly turbid but this does not interfere.
6. Prepare a 100 µM stock of standard by diluting 1 µL of the 100 mM Standard with 999 µL of dH2O, then dilute in Assay Buffer to give 100, 60, 30 and 0 µM standards (fluorimetric) or 0, 300, 600 and 1000 µM standards (colorimetric).
7. Transfer 10 µL of each standard into separate wells of a black 96-well plate (fluorimetric) or clear 96-well plate (colorimetric), and transfer 10 µL of each sample into separate wells.
8. For samples requiring an internal standard, run three separate reactions per sample - Sample plus Standard, Sample alone and Sample Blank - adding 1 µL of 1 mM Standard to 39 µL of sample for the Sample plus Standard well.
9. 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, then add 90 µL to each well, tap to mix and incubate at room temperature for 30 min protected from light.
10. If using an internal standard, prepare a Sample Blank Working Reagent with all components except Enzyme A and add 90 µL to the Sample Blank wells.
11. Read fluorescence (Ex/Em = 530/585 nm) or optical density (570 nm) immediately (t0min) and at 30 min (t30min), or record kinetics for 30 min.

Standard Preparation (Fluorimetric Assay)

No	100 µM STD + dH2O	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

Calculation

For samples without background matrix effects, use values obtained at 30 min: subtract the Blank value (Standard #4) from the standard values, plot dOD or dF against standard concentrations, determine the slope and calculate the sample L-Carnitine concentration. For samples with background matrix effects (e.g. milk and serum), an internal standard should be used, calculated from dR_S, dR_SB and dR_IS (the readings of the Sample, Sample Blank and Sample plus Internal Standard, respectively, at t30min - t0min) and the dilution factor n. If the standard curve is non-linear, use polynomial fitting to solve for the concentration.

Notes

- The colorimetric assay procedure is similar to the fluorimetric procedure except that 0, 300, 600 and 1000 µM standards and a clear 96-well plate are used, reading optical density at 570 nm.
- 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 the sample in dH2O and repeat the assay, then multiply the result by the dilution factor n.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.

Technical specifications are based on manufacturer documentation and may be updated. Protocols shown are summaries - always follow the instructions supplied with your kit/lot.