Technical Bulletin

L-Carnitine Assay Kit

Catalogue Number MAK628

Product Description

L-Carnitine is a naturally occurring compound biosynthesized from lysine and methionine that is essential for cellular energy metabolism, with the highest concentrations in skeletal and cardiac muscle. Its primary function is facilitating the transport of long-chain fatty acids into the mitochondrial matrix for β-oxidation via the carnitine shuttle. Beyond transport, L-carnitine serves a critical acyl-group buffering function by reversibly forming acylcarnitines of varying chain lengths, which maintains free CoA availability for essential metabolic reactions, enables export of excess or toxic acyl intermediates, and generates metabolite signatures that reflect the balance between substrate supply, oxidative capacity, and metabolic bottlenecks. Carnitine metabolism is a central hub connecting fatty acid oxidation, mitochondrial function, branched-chain amino acid catabolism, and cellular energy homeostasis across physiological and disease states.

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 λex/em = 530/585 nm is directly proportional to the L-Carnitine concentration in the sample. Detection range: colorimetric assay 12 - 1000 µM, fluorometric assay 1 - 100 µM L-Carnitine.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK628A
- Enzyme A 1 vial
  Catalogue Number MAK628B
- Enzyme B 100 µL
  Catalogue Number MAK628C
- Enzyme C 100 µL
  Catalogue Number MAK628D
- Substrate Mix 400 µL
  Catalogue Number MAK628E
- Dye Reagent 120 µL
  Catalogue Number MAK628F
- Standard 50 µL
  Catalogue Number MAK628G

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Plate reader capable of λex/em = 530/585 nm or OD 570nm.
- Clear plates for colorimetric assays (Catalogue number M2936 or equivalent).

  Black plates with clear bottoms for fluorescence assays (Catalogue number CLS3631 or equivalent).

  Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. Store at -20°C upon receipt.

Preparation Instructions

Samples can be analyzed immediately after collection or stored in aliquots at –20°C. Avoid repeated freeze-thaw cycles.

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 14000 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 14000 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.

Fluorometric Standard Preparation

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:

Table 1.
Fluorometric Standard Preparation

Std #	Standard Premix (µL)	Water (µL)	Conc. (µM)
1	100	0	100
2	60	40	60
3	30	70	30
4	0	100	0

Colorimetric Standard Preparation

Prepare a 1000 µM stock of standard by diluting 10 µL of the 100 mM Standard with 990 µL of dH_2O. Dilute the 1000 µM standard in Assay Buffer as follows:

Table 2.
Colorimetric Standard Preparation

Std #	Standard Premix (µL)	Water (µL)	Conc. (µM)
1	100	0	1000
2	60	40	600
3	30	70	300
4	0	100	0

Procedure

Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay.

Note: The Substrate Mix may be slightly turbid, but the turbidity does not interfere with the reaction.

Use black flat-bottom 96-well plates for fluorometric method.

Use clear 96-well plates for colorimetric method.

Assay Reaction

1. Transfer 10 µL of each standard into separate wells of a 96-well plate.
2. 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
   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. 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.
4. Add 90 µL of WR to each well, tap to mix and incubate at room temperature (RT) for 30 min protected from light.
5. 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.
6. Fluorometric: Read fluorescence (λex/em = 530/585 nm) immediately (t_{0min}) and at 30 min (t_{30min}), or record kinetics for 30 min.

   Colorimetric: Read optical density at 570 nm immediately (t_{0min}) and at 30 min (t_{30min}), or record kinetics for 30 min.

Results

Calculations

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*.

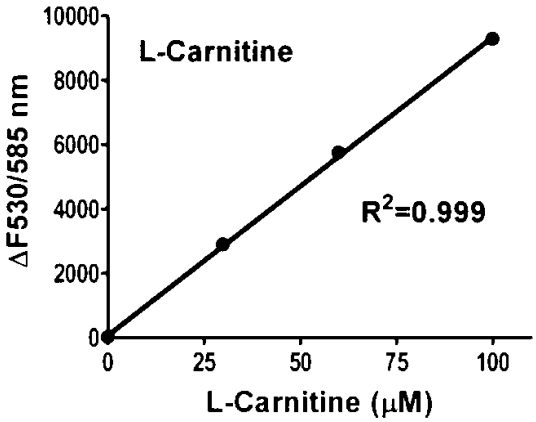

Figure 1.
Exemplary fluorometric standard curve

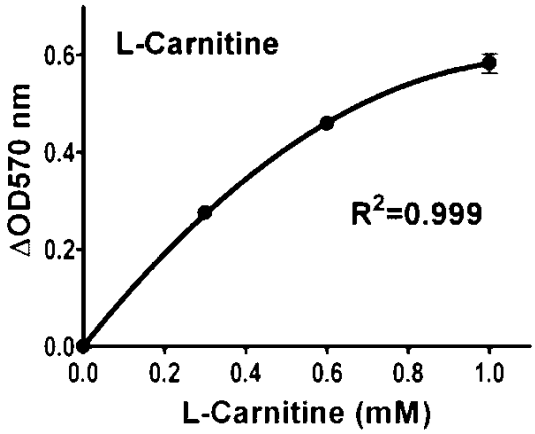

Figure 2.
Exemplary colorimetric standard curve

References

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.

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