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Product Information

L-Carnitine Assay Kit

Catalog Number **MAK063**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Carnitine is a metabolite synthesized from lysine and methionine. Carnitine is essential for fatty acid β-oxidation due to its role in fatty acid transport into the mitochondrial matrix via the carnitine/acylcarnitine shuttle. Carnitine exists in two stereoisomers but only the L-carnitine isomer is biologically active. Tissue levels of L-carnitine decrease with aging and decreased L-carnitine may contribute to age-related mitochondrial decline.

The L-Carnitine Assay Kit is a simple convenient means of measuring free L-Carnitine in biological samples such as serum. L-carnitine concentration is determined by a coupled enzyme assay, which results in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product, proportional to the L-carnitine present. Typical detection range for this kit is 2–10 nmoles (colorimetric) and 0.2–1 nmoles (fluorometric).

Components

The kit is sufficient for 100 assays in 96 well plates.

Carnitine Assay Buffer Catalog Number MAK063A	25 mL
Carnitine Probe, in DMSO Catalog Number MAK063B	0.2 mL
Carnitine Converting Enzyme Catalog Number MAK063C	1 vl
Carnitine Substrate Mix, in DMSO Catalog Number MAK063D	0.4 mL
Carnitine Development Mix Catalog Number MAK063E	1 vl
Carnitine Standard, 10 µmole Catalog Number MAK063F	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader.
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter (optional for serum samples)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Carnitine Assay Buffer – Allow buffer to come to room temperature before use.

Carnitine Probe – Warm probe to room temperature to melt DMSO prior to use. Mix well by pipetting, then aliquot and store, protected from light and moisture, at –20 °C.

For the fluorescence assay, dilute an aliquot of the Carnitine Probe Solution 5 to 10-fold with Carnitine Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Carnitine Converting Enzyme Mix and Carnitine Development Mix – Reconstitute in 220 µL of Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light and moisture, at –20 °C. Use within 2 months of reconstitution.

Carnitine Substrate Mix – Ready to use as supplied. Warm to room temperature to melt DMSO prior to use. Will show cloudiness, which does not interfere with assay.

Carnitine Standard - Reconstitute in 100 µL of water to generate a 100 mM (100 nmole/µL) solution. Mix well by pipetting, then aliquot and store, protected from light and moisture, at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure

Carnitine Standards for Colorimetric Detection

Dilute 10 µL of the 100 mM carnitine standard with 990 µL of water to prepare a 1 mM carnitine standard solution. Add 0, 2, 4, 6, 8, and 10 µl of the 1 mM carnitine standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Carnitine Assay Buffer to each well to bring the volume to 50 µL.

Carnitine Standards for Fluorometric Detection

Prepare a 1 mM standard solution as for the colorimetric assay. Dilute 10 µL of the 1 mM standard solution with 90 µL of the Carnitine Assay Buffer to make a 0.1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 mM standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Carnitine Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation

Both the colorimetric and fluorometric assays require 50 µL of sample for each reaction (well).

Tissue or cells (1×10^6) can be homogenized in 100 µL of the Carnitine Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material. If enzymes present in the sample interfere with the assay, samples can be deproteinized with a 10 kDa MWCO spin filter.

The normal range for serum L-Carnitine is between 10–70 µM. Serum samples should be deproteinized before with a 10 kDa MWCO spin filter.

Bring samples to a final volume of 50 µL with Carnitine Assay Buffer. For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Assay Reaction

1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).
   Note: Acyl-CoA or free Coenzyme A in samples can generate background readings. To remove the Acyl-CoA or free Coenzyme A background, include a blank sample for each sample by omitting the Carnitine Converting Enzyme Mix. The blank control readings can then be subtracted from the sample readings.

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Blank Sample
Carnitine Assay Buffer	40 µL	42 µL
Carnitine Converting Enzyme	2 µL	–
Carnitine Development Mix	2 µL	2 µL
Carnitine Substrate Mix	4 µL	4 µL
Carnitine Probe (colorimetric or fluorescence)	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.

3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for either assay is the value obtained for the 0 (blank) Carnitine standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate Carnitine standards to plot a standard curve. The amount of carnitine present in the samples may be determined from the standard curve.

Note: A new standard curve must be set up each time the assay is run.

Concentration of L-Carnitine

$$S_a/S_v = C$$

S_a = Amount of carnitine in unknown sample (nmole) from standard curve

S_v = Sample volume (µL) added into the wells.

C = Concentration of carnitine in sample

L-Carnitine molecular weight: 161.2 g/mole

Sample Calculation

Amount of Carnitine (S_a) = 5.84 nmole

Sample volume (S_v) = 50 µL

Concentration of Carnitine in sample

$$5.84 \text{ nmole}/50 \ \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 161.2 \text{ ng/nmole} = 18.83 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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