Product Information

L-Carnitine Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2142-100 100 assays	Cap Color	Part Number
Carnitine Assay Buffer	25 ml	WM	K2142-C-1
Carnitine Probe (in DMSO)	0.2ml	Red	K2142-C-2
Carnitine Converting Enzyme	lyophilized	Purple	K2142-C-3
Carnitine Substrate Mix	400 µl	Blue	K2142-C-4
Carnitine Development Mix	lyophilized	Green	K2142-C-5
Carnitine Standard (10 µmol)	lyophilized	Yellow	N1935

Mixture of all amino acids at equal molar ratio. Glycine is not detectable in this assay

II. Introduction:

Carnitine is a quaternary ammonium compound produced from methionine and lysine. Carnitine plays an important role in transporting fatty acids into the mitochondrial matrix via the carnitine/acylcarnitine shuttle where occurs β-oxidation, acetate is produced and is utilized to generate energy in the TCA cycle. Carnitine exists in two isomers. Only L-carnitine is biologically active. L-Carnitine is often considered as a nutritional supplement.

The L-Carnitine Colorimetric/Fluorometric Assay Kit provides a simple, fast and convenient way for detection of free L-carnitine in various biological samples based on colorimetric and fluorometric method. In the assay, CoA transfers an acetyl group to carnitine and the free CoA produced is further processed with subsequent oxidation of the Oxi-Red probe to give absorbance (570 nm) and fluorescence (Ex/Em 535 nm 587 nm). The normal range of L-carnitine in serum is ~ 20 - 100 µM. The detection sensitivity is ~ 10 µM for the colorimetric assay and ~ 1 µM for the fluorometric assay.

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

Carnitine Probe: Ready to use as supplied. Warm to room temperature to melt frozen DMSO before use. Protect from light and moisture. Stable for 2 months at -20°C.

Carnitine Converting Enzyme, Development Mix: Dissolve with 220 µl Carnitine Assay Buffer separately. Pipette up and down to dissolve. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months.

Carnitine Substrate Mix: Ready to use as supplied. Bring to room temperature to melt frozen DMSO. Will show cloudiness which does not interfere with the assay.

Carnitine Standard: Dissolve in 100 µl dH_2O to generate 100 mM (100 nmol/µl) Carnitine Standard solution. Keep on ice while in use. Store at -20°C. Keep the Enzyme and Development Mix on ice during the assay and protect from light. Ensure that the Assay Buffer is warmed to room temperature before use.

V. Carnitine Assay Protocol:

1. Carnitine Standard Curve:

For the Colorimetric Assay: Dilute 10 µl of the 100 mM Carnitine Standard with 990 µl dH_2O to generate 1 mM standard Carnitine. Add 0, 2, 4, 6, 8, 10 µl of the diluted Carnitine Standard into a 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well standard. Bring the volume to 50 µl with Assay Buffer.

For the Fluorometric Assay: Dilute the standard to 0.1 mM (0.1 nmol/µl), then follow the same protocol as colorimetric assay. Will give 0, 0.2, 0.4, 0.6, 0.8, 1 nmol/well

2. Sample Preparation: Tissues or cells (1×10^6) can be homogenized in 100 µl Assay Buffer and centrifuged to remove insoluble materials at 13,000 g for 10 min. 10-50 µl deproteinized serum samples can be directly diluted in the Assay Buffer. Bring sample wells to 50 µl/well with Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to make sure the readings are within the standard curve range. Deproteinization may be done by PCA precipitation followed by KOH neutralization or using centrifugation through a 10kDa MW cut-off filter . The normal range for serum L-carnitine is ~ 10 - 70 µM.

3. Reaction Mix: Mix enough reagent for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

	L-Carnitine Measurement	Background Control
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	2 µl	2 µl

Perform background control if high levels of acyl -CoA's or free Coenzyme A are suspected to be in your samples. Choline in samples will give a positive signal but is present at ~ 10% of the Carnitine concentration.

For the fluorescent assay, dilute the probe 10X to reduce background reading. Add 50 µl of the Reaction Mix to each well containing the Carnitine standard, test and background control samples. Mix well. Incubate the reaction for 30 min at room temperature, protect from light.

4. Measure OD at 570 nm, or fluorescence at Ex/Em 535/587 nm in a microplate reader.

5. Calculation: Correct background by subtracting the value derived from the 0 Carnitine control from all sample and standard readings (The background reading can be significant and must be subtracted from sample readings). Plot Carnitine standard curve. Apply sample readings to the standard curve. Carnitine concentrations of the test samples can then be calculated:

$C = Sa/Sv$ (nmol/µl, or mM)

where Sa is the Carnitine content of unknown samples (in nmol) from standard curve,

Sv is sample volume µl) added into the assay wells.

L-Carnitine Molecular Weight is 161.2 g/mol.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet

	• Samples were not deproteinized (if indicated in d atasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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