

L-Carnitine Assay Kit

(Catalog #K642-100; 100 Reactions; Store kit at -20°C)

I. Introduction:

Carnitine is a quaternary ammonium compound biosynthesized from lysine and methionine. It is required for fatty acid transport into the mitochondrial matrix via the carnitine/acylcarnitine shuttle, where β -oxidation occurs, acetate is generated and the acetate utilized in the TCA cycle for the generation of energy. Carnitine exists in two stereoisomers. Only L-carnitine is biologically active. BioVision's L-Carnitine Assay Kit is a simple convenient means of measuring free L-Carnitine in biological samples such as serum. The assay transfers an acetyl group from CoA to Carnitine and the free CoA formed is further processed with subsequent oxidation of the Oxi-Red probe to give fluorescence (Ex/Em 535/587 nm) and absorbance (570 nm). The normal range for serum L-Carnitine is ~10-70 μ M. The detection sensitivity is ~1 μ M (fluorometric) and ~10 μ M (colorimetric).

II. Kit Contents:

Components	K642-100	Cap Code	Part No.
Carnitine Assay Buffer	25 ml	WM	K642-100-1
Carnitine Probe (in DMSO)	0.2ml	Red	K642-100-2A
Carnitine Converting Enzyme	lyophilized	Purple	K642-100-4
Carnitine Substrate Mix	400 μ l	Blue	K642-100-5
Carnitine Development Mix	lyophilized	Green	K642-100-6
Carnitine Standard (10 μ mol)	lyophilized	Yellow	K642-100-7

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 μ l 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 μ l dH₂O to generate 100 mM (100 nmol/ μ l) 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 μ l of the 100 mM Carnitine Standard with 990 μ l dH₂O to generate 1 mM standard Carnitine. Add 0, 2, 4, 6, 8, 10 μ l 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 μ l with Assay Buffer.

For the Fluorometric Assay: Dilute the standard to 0.1 mM (0.1 nmol/ μ l), 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 (1x10⁶) can be homogenized in 100 μ l Assay Buffer and centrifuged to remove insoluble materials at 13,000 g for 10 min. 10-50 μ l deproteinized serum samples can be directly diluted in the Assay Buffer. Bring sample wells to 50 μ l/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 (BioVision K808-200) or using centrifugation through a 10kDa MW cut-off filter (BioVision part 1997-25). The normal range for serum L-carnitine is ~ 10-70 μ M.

3. Reaction Mix:

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

	L-Carnitine Measurement	Background Control*
Assay Buffer	40 μ l	42 μ l
Carnitine Converting Enzyme	2 μ l	-----
Carnitine Development Mix	2 μ l	2 μ l
Carnitine Substrate Mix	4 μ l	4 μ l
Carnitine Probe**	2 μ l	2 μ l

* 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.

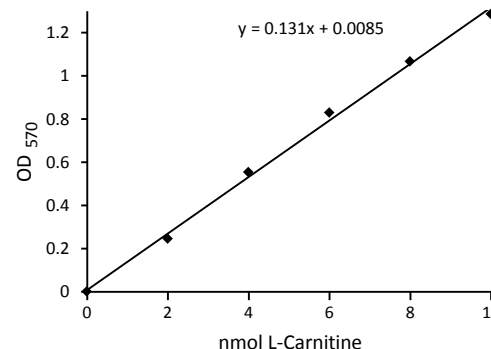
Add 50 μ l of the **Reaction Mix** to each well containing the Carnitine standard, test and background control samples. Mix well. Incubate the reaction for 30 min at room temperature, protect from light.

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:

$$C = S_a/S_v \text{ (nmol/}\mu\text{l, or mM)}$$

where S_a is the Carnitine content of unknown samples (in nmol) from standard curve, S_v is sample volume (μ l) added into the assay wells. L-Carnitine Molecular Weight is 161.2 g/mol.



RELATED PRODUCTS:

NAD(P)/NAD(P)H Quantification Kit	ADP/ATP Ratio Assay Kit
Ascorbic Acid Quantification Kit	Glutathione Detection Kit
Total Antioxidant Capacity (TAC) Assay Kit	Fatty Acid Assay Kit
Ethanol Assay Kit	Uric Acid Assay Kit
Pyruvate Assay Kit	Lactate Assay Kit I & II
Creatinine Assay Kit	Nitric Oxide Assay Kit
Ammonia Assay Kit	Free Glycerol Assay Kit
Triglyceride Assay Kit	Hemin Assay Kit
Choline/Acetylcholine Quantification Kit	Glucose Assay Kit
Sarcosine Assay Kit	L-Amino Acid Assay Kit
Glycogen Assay Kit	Cholesterol Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • 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 (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • 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 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet 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	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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 datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • 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
<p>Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</p>		