

L-Carnitine Assay Kit

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

I. Introduction:

Carnitine is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine. It is required for transport of fatty acids 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. L-Carnitine is often sold as a nutritional supplement. 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 nm 587 nm) and absorbance (570 nm). The normal range for serum L-Carnitine is ~20-100 μ M. The detection sensitivity is ~1 μ M for the fluorometric assay and ~10 μ M for the colorimetric assay.

II. Kit Contents:

Components	K642-100	Cap Code	Part No.
Carnitine Assay Buffer	25 ml	WM	K642-100-1
Carnitine Probe	lyophilized	Red	K642-100-2
DMSO, Anhydrous	400 μ l	Brown	K642-100-3
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: Add 220 μ l DMSO. Pipette up and down to dissolve. 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: Bring to room temperature to melt DMSO. Ready to use as supplied. 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 Preparations:

Tissues or cells (1×10^6) can be homogenized in 100 μ l Assay Buffer, centrifuge to remove insoluble materials at 13,000 g for 10 minutes. 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 10kD MW cut-off filter (BioVision part 1997-25).

3. Reaction Mix:

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

L-Carnitine Measurement

40 μ l Assay Buffer
2 μ l Carnitine Converting Enzyme
2 μ l Carnitine Development Mix
4 μ l Carnitine Substrate Mix
2 μ l Carnitine Probe**

Background Control*

42 μ l Assay Buffer

2 μ l Carnitine Development Mix
4 μ l Carnitine Substrate Mix
2 μ l Carnitine Probe

* 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.

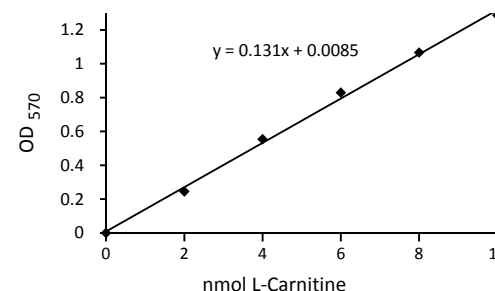
Add 50 μ l of the **Reaction Mix** to each well containing the Carnitine standard and test samples. Mix well. Incubate the reaction for 30 min at room temperature, protect from light.

4. Measure O.D. 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 Alanine control from all sample and standard readings (The background reading can be significant and must be subtracted from sample readings). Plot Alanine standard Curve. Apply sample readings to the standard curve. Alanine concentrations of the test samples can then be calculated:

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

where S_s 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.



VI. Related Products:

NAD(P)/NAD(P)H Quantification Kit
Ascorbic Acid Quantification Kit
Total Antioxidant Capacity (TAC) Assay Kit
Ethanol Assay Kit
Pyruvate Assay Kit
Creatinine Assay Kit
Ammonia Assay Kit
Triglyceride Assay Kit
Choline/Acetylcholine Quantification Kit
Sarcosine Assay Kit
Glycogen Assay Kit

ADP/ATP Ratio Assay Kit
Glutathione Detection Kit
Fatty Acid Assay Kit
Uric Acid Assay Kit
Lactate Assay Kit I & II
Nitric Oxide Assay Kit
Free Glycerol Assay Kit
Hemin Assay Kit
Glucose Assay Kit
L-Amino Acid Assay Kit
Cholesterol Assay Kit