L-Carnitine Colorimetric/Fluorometric Assay Kit (#BN00866)

(Catalog #BN00866; 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. Assay Genie'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 µM. The detection sensitivity is ~1 µM (fluorometric) and ~10 µM (colorimetric).

II. Kit Contents:

Components	BN00866	Cap Code	Part No.
Carnitine Assay Buffer	25 ml	WM	BN00866-1
Carnitine Probe (in DMSO)	0.2ml	Red	BN00866-2A
Carnitine Converting Enzyme	lyophilized	Purple	BN00866-4
Carnitine Substrate Mix	400 µl	Blue	BN00866-5
Carnitine Development Mix	lyophilized	Green	BN00866-6
Carnitine Standard (10 µmol)	lyophilized	Yellow	BN00866-7

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 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.

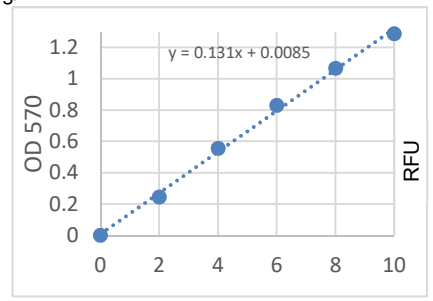
   Add 50 µl of the **Reaction Mix** to each well containing the Carnitine standard, test and background control samples. Mix well. Incubate the reaction for 30 min at room temperature, protect from light.

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

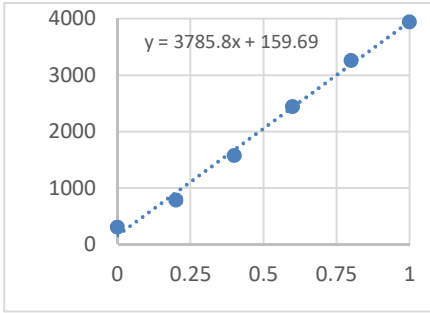
5. **Calculation:** Correct background by subtracting the value derived from the 0 Carnitine control from all sample and standard readings (The background reading can be significant and must be subtracted from sample readings). Plot Carnitine standard curve. Apply sample readings to the standard curve.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

   $C = S_a/S_v$ (nmol/µl, or mM)

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



nmol L-carnitine



nmol L-carnitine

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

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till 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	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		