MA-0161

L-Carnitine Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Introduction:

Carnitine is a critical transport molecule which shuttles fatty acids into the mitochondria where they are utilized to make acetyl CoA, a key part of the TCA cycle. In the reverse direction carnitine shuttles toxic by-products of fatty acid metabolism out of the mitochondria, minimizing oxidative stress and oxidative damage that would otherwise occur. L-Carnitine is synthesized from methionine and lysine. Beyond its highly important shuttle function, it also enhances insulin sensitivity and cellular glucose uptake. AkrivisBio's L-Carnitine Assay is a simple, sensitive means of measuring free L-Carnitine in biological samples.

Assay Principle:

- Carnitine is acetylated by acetyl CoA via Carnitine acetyl transferase.
- The free CoA is esterified with a fatty acid which is subsequently oxidized with the formation of hydrogen peroxide.
- Peroxidase utilizes hydrogen peroxide to oxidize ADHP to resorufin with a dramatic increase in color and fluorescence.

Assay Components:

Assay Buffer	25 ml	WM	MA-0161A
ADHP Solution	0.2ml	Red	MA-0161B
Carnitine Acetyltransferase	lyoph	Purple	MA-0161C
Acetyl CoA	400 µl	Blue	MA-0161E
Carnitine Development Mix	lyoph	Green	MA-0161F
L-Carnitine Standard	lyoph	Yellow	MA-0161G

Storage and Handling Considerations:

Store the unopened assay at -20°C. Bring all components to room temperature before use. Centrifuge all vials for a few seconds before opening.
Assay Buffer: Ready to use as supplied. Store at 4°C.
ADHP Solution: Ready to use as supplied. Light and moisture sensitive, so keep closed. Store at -20°C.
Carnitine Acetyltransferase, Carnitine Development Mix: Dissolve each with 220 µl Carnitine Assay Buffer. Aliquot into convenient portions and store at -20°C. Keep on ice while in use.
Acetyl CoA: Ready to use as supplied. Can show cloudiness which does not interfere with the assay.
Carnitine Standard: Dissolve in 100 µl DI H_2O giving a 40 mM Carnitine solution. Keep on ice while in use. Store at -20°C.

Carnitine Assay Protocol:

1. Standard Curve:

Absorbance-based Assay: Add 10 µl of the 40 mM Carnitine solution to 990 µl DI H_2O giving 0.4 mM Carnitine. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of the 0.4 mM Carnitine Standard to a 96-well plate giving 0, 2, 4, 6, 8, 10 nmol of the Standard. Adjust the wells to 50 µl with Assay Buffer.

Fluorescence-based Assay: Dilute as above, then dilute a further 10X by adding 10 µl to 90 µl Assay Buffer Transfer 0 – 5 – 10 – 15 – 20 – 25 µl to a 96-well plate giving 0, 0.2, 0.4, 0.6, 0.8 and 1 nmol respectively.

2. Sample Preparation:

Homogenize tissue (10 mg) or cells (1×10^6) in 100 µl Assay Buffer and centrifuge to remove insoluble material (16,000 X g, 10 min). Transfer the clear supernatant to a fresh tube. Up to 50 µl of supernatant or deproteinized serum can be added directly to a 96-well plate. Adjust wells to 50 µl with Assay Buffer.

Note: High levels of acyl CoA's or free Coenzyme A will cause significant background. Run samples in pairs if these are expected to be in your samples with one well of the pair being used as a Background Control well.

3. Initiate Reaction:

Each well will require 50 µl of Reaction Mix. Prepare sufficient Reaction Mix for the total number of wells to be analyzed, containing:

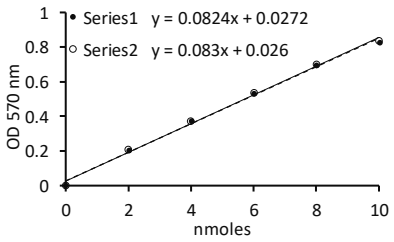
	Reaction Mix	Background Control
Assay Buffer	40 µl (41.8 µl)*	42 µl (41.8 µl)*
Carnitine Acetyltransferase	2 µl	------
Carnitine Development Mix	2 µl	2 µl
Acetyl CoA	4 µl	4 µl
ADHP Solution	2 µl (0.2 µl)*	2 µl (0.2 µl)*

* For the fluorescent assay, dilute the probe 10X to reduce background reading.
Add 50 µl of Reaction Mix to each Standard, Sample or Background Control well.

4. Measure: Monitor the reaction using absorbance at 570 nm, or fluorescence at Ex/Em 535/587 nm. Acquire data in kinetic mode when possible.

5. Typical Results:

nmol Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0914	0.0948	0	0
2	0.2985	0.3016	0.2071	0.2068
4	0.4632	0.4664	0.3718	0.3716
6	0.6244	0.6295	0.5330	0.5347
8	0.7868	0.7925	0.6954	0.6977
10	0.9201	0.9295	0.8287	0.8346



6. Calculation: Subtract the zero-standard reading from all of the other standards. Plot the Standard Curve. Determine the slope of the Standard Curve. Subtract any Background Control well values from their paired samples well readings. If there is no paired Background Control well for a sample, subtract the zero-standard value. Divide the background corrected sample readings by the slope of the Standard Curve to obtain nmoles of L-Carnitine in the well. To convert back to L-carnitine in the original sample:
- Divide the nmoles of carnitine in the well by the volume of sample applied to the well = nmoles of carnitine per µl sample
- Multiply the nmoles of carnitine per µl sample X the total volume of supernatant obtained in step 2 above = total nmoles carnitine per sample.
- Divide the total nmoles carnitine per sample by the mg tissue or # of cells used = nmole carnitine per mg tissue (per # of cells) used.

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