ab83392

L-Carnitine Quantification Assay kit (Colorimetric/Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of L-carnitine in various samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 6 Last Updated 23 August 2023

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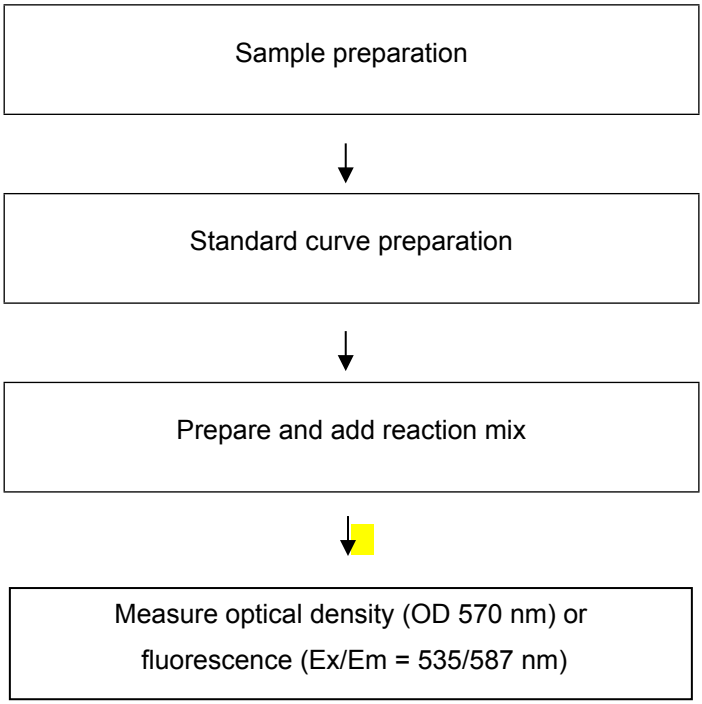
1. BACKGROUND

L-Carnitine Assay Kit (colorimetric/fluorometric) (ab83392) 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 OxiRed probe to give fluorescence (Ex/Em 535/587 nm) and absorbance (570 nm). The normal range for serum L-Carnitine is ~20-100 µM. The detection sensitivity is ~1 µM for the fluorometric assay and ~10 µM for the colorimetric assay.

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. Carnitine exists in two stereoisomers. Only L-carnitine is biologically active.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature**. Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Carnitine Assay Buffer	25 mL	-20°C	-20°C
OxiRed Probe/Carnitine Probe (in DMSO)	200 µL	-20°C	-20°C
Carnitine Converting Enzyme (Lyophilized)	50 µL	-20°C	-20°C
Carnitine Substrate Mix	400 µL	-20°C	-20°C
Carnitine Development Mix (Lyophilized)	1 vial	-20°C	-20°C
Carnitine Standard (10 µmol) (Lyophilized)	1 vial	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Colorimetric or fluorescent microplate reader – equipped with filter for OD 570 nm or Ex/Em = 535/587 nm (respectively)
- 96 well plate: black plates (clear bottoms) for fluorometric assay; clear plates for colorimetric assay
- Microcentrifuge
- Pipettes and pipette tips
- Vortex
- Dounce homogenizer (if using tissue)
- Orbital shaker

If performing deproteinization step, additional reagents are required:

- Perchloric acid (PCA) 4M, ice cold
- Potassium Hydroxide (KOH) 2M

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Carnitine Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **OxiRed Probe/Carnitine Probe:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** Store at -20°C protected from light. Once the OxiRed Probe/probe is thawed, use with two months.

9.3 **Carnitine Converting Enzyme:**

Dissolve with 220 µL Carnitine Assay Buffer. Pipette up and down to dissolve. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use. Use within two months.

9.4 **Carnitine Substrate Mix:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when let at room temperature, so it needs to melt for few minutes at 37°C.** Aliquot OxiRed Probe/probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Once thawed, use with two months.

Will show cloudiness which does not interfere with the assay. Keep on ice while in use.

9.5 **Carnitine Development Mix:**

Dissolve with 220 µL Carnitine Assay Buffer. Pipette up and down to dissolve. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use. Use within two months.

9.6 **Carnitine Standard:**

Dissolve in 100 µL dH_2O to generate 100 mM (100 nmol/ µL) Carnitine Standard solution. Store at -20°C. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 For the colorimetric assay

10.1.1 Prepare a 1 mM Carnitine standard by diluting 5 µL of the 100 mM standard in 495 µL ddH_2O.

10.1.2 Using 1 mM Carnitine standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of 1mM Carnitine Standard (µL)	Assay Buffer (µL)	Final volume in well (µL)	End [Carnitine] in well
1	0	150	50	0 nmol/well
2	6	144	50	2 nmol/well
3	12	138	50	4 nmol/well
4	18	132	50	6 nmol/well
5	24	126	50	8 nmol/well
6	30	120	50	10 nmol/well

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL).

10.2 **For the flurometric assay:**

10.2.1 Prepare a 1 mM carnitine standard by diluting 5 µL of the 100 mM standard in 495 µL ddH_2O.

10.2.2 Prepare a 0.1 mM (0.1 nmol/µL) carnitine standard by diluting 100 µl of the 1mM standard in 900 µL dH_20.

10.2.3 Using 0.1mM Carnitine standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of 0.1mM Standard (µL)	Assay Buffer (µL)	Final volume in well (µL)	End [Carnitine] in well
1	0	150	50	0 nmol/well
2	6	144	50	0.2 nmol/well
3	12	138	50	0.4 nmol/well
4	18	132	50	0.6 nmol/well
5	24	126	50	0.8 nmol/well
6	30	120	50	1.0 nmol/well

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL).

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 **Cell (adherent or suspension) samples:**

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL Assay Buffer.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Centrifuge 2 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.2 **Tissue Samples:**

11.2.1 Harvest the necessary amount of tissue for each assay (initial recommendation = 10 mg tissue).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 500 – 1,000 µL (or approximately 4-6 volumes) of Assay Buffer.

11.2.4 Centrifuge for 10 minutes at 13,000 x g using a microcentrifuge to remove any insoluble material.

11.2.5 Keep on ice.

11.3 **Plasma, Serum and Urine and other biological fluids:**

10 – 50 µL deproteinized serum samples can be directly diluted in the Assay Buffer.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (1:2 – 1:5 – 1:10).

The normal range for serum L-carnitine is ~10-70 µM.

11.4 **Deproteinization protocol**

Prepare samples as specified in section 11.1, 11.2 or 11.3. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.

11.4.1 Add ice cold PCA to a final concentration of 1 M in the homogenate solution and vortex briefly to mix well. ***NOTE:*** *high protein concentration samples might need more PCA.*

11.4.2 Incubate on ice for 5 minutes.

11.4.3 Centrifuge samples at 13,000 x g for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube.

11.4.4 Precipitate excess PCA by adding ice-cold 2 M KOH that equals 34% of the supernatant to your samples (for instance, 34 µL of 2 M KOH to 100 µL sample) and vortexing briefly. This will neutralize the sample and precipitate excess PCA. There may be some gas (CO_2) evolution so vent the sample tube.

11.4.5 After neutralization, it is very important that pH equals 6.5 – 8 (use pH paper to test 1 µL of sample). If necessary, adjust the pH with 0.1 M KOH.

11.4.6 Centrifuge at 13,000 x g for 15 minutes at 4°C and collect supernatant.

11.4.7 Transfer supernatant to a clean tube, and keep on ice.

Samples are now deproteinized, neutralized and PCA has been removed. The samples are now ready to use in the assay.

Sample Recovery

The deproteinized samples will be diluted from the original concentration.

To calculate the dilution factor of your final sample, simply apply the following formula:

$$\%\ original\ concentration = \frac{a}{a+b} \times \frac{c}{c+d} \times 100$$

a = initial sample volume

b = PCA volume

c = Supernatant volume

d = KOH volume

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **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.**

12.1 **Set up Reaction wells**:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 10-50 µL (adjust volume to 50 µL/well with Assay Buffer).
- Background wells = 50 µL Assay Buffer.
- (Optional) Sample background control = 10-50 µL (adjust volume to 50 µL/well with Assay Buffer).

12.2 **Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction.

Component	Colorimetric Reaction Mix (µL)	Background Control (µL)*
Assay Buffer	**40**	**42**
Carnitine Converting Enzyme	**2**	**0**
Carnitine Development Mix	**2**	**2**
Carnitine Substrate Mix	**4**	**4**
OxiRed Probe/Carnitine Probe	**2**	**2**

For fluorometric readings, using 0.2 µL/well of the OxiRed Probe/probe decreases the background readings, therefore increasing the detection sensitivity:

Component	Fluorescent Reaction Mix (µL)	Background Control (µL)*
Assay Buffer	**41.8**	**43.8**
Carnitine Converting Enzyme	**2**	**0**
Carnitine Development Mix	**2**	**2**
Carnitine Substrate Mix	**4**	**4**
OxiRed Probe/Carnitine Probe	**0.2**	**0.2**

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency.

We recommend the following calculation:

X µL component x (Number samples + Standards +1)

12.3 Add 50 µL of Reaction Mix into each well containing the standard, sample and background control samples. Mix well.

12.4 Incubate the reaction at room temperature for 30 minutes protected from light.

12.5 Measure output on a microplate reader:

- Colorimetric assay: measure OD 570 nm.
- Fluorometric assay: measure Ex/Em = 535/587 nm.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance. The background reading can be significant and must be subtracted from sample readings).

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of Carnitine.

13.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Extrapolate sample readings from the standard curve plotted using the following equation:

$$x = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.6 Concentration of L-carnitine (as nmol/µL or mM) in the test samples is calculated as:

$$L - carnitine\ concentration = \left(\frac{Sa}{Sv}\right) * D$$

Where:

Sa = Carnitine content of unknown samples (in nmol)

Sv = Sample volume added in sample wells (µL).

D = Dilution factor.

L-Carnitine molecular weight is 161.2 g/mol.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

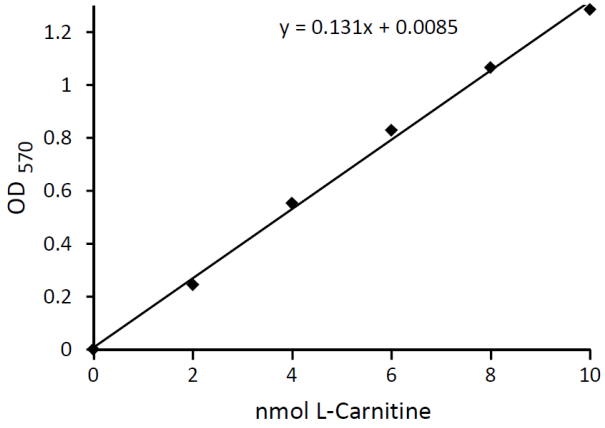
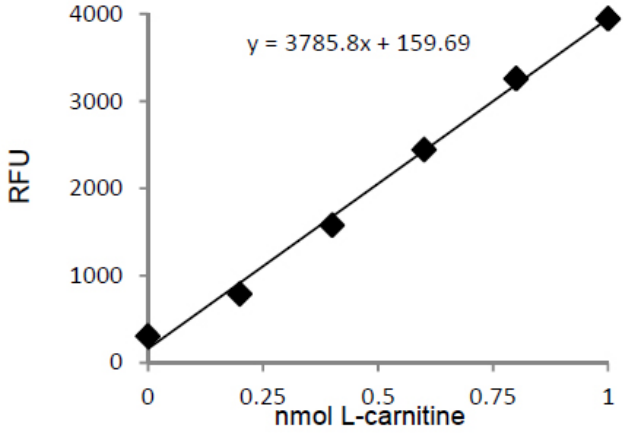


Figure 1. Typical L-Carnitine standard calibration curve obtained using



colorimetric reading.

Figure 2. Typical L-Carnitine standard calibration curve obtained using fluorometric ready.

15. QUICK ASSAY PROCEDURE

NOTE: *This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Solubilize standard, OxiRed Probe/probe, converting enzyme, substrate and development mix; (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL).
- Prepare Reaction Mix (Number samples + standards + 1).

Component	Colorimetric Reaction Mix (µL)	Background Control (µL)*
Assay Buffer	**40**	**42**
Carnitine Converting Enzyme	**2**	**0**
Carnitine Development Mix	**2**	**2**
Carnitine Substrate Mix	**4**	**4**
OxiRed Probe/Carnitine Probe	**2**	**2**

Component	Fluorescent Reaction Mix (µL)	Background Control (µL)*
Assay Buffer	**41.8**	**43.8**
Carnitine Converting Enzyme	**2**	**0**
Carnitine Development Mix	**2**	**2**
Carnitine Substrate Mix	**4**	**4**
OxiRed Probe/Carnitine Probe	**0.2**	**0.2**

- Add 50 µL of appropriate Reaction Mix to wells.
- Incubate plate RT 30 mins.
- Measure plate at OD 570 nm for colorimetric assay or Ex/Em = 535/587 nm for fluorometric assay.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

Can we use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot experiment with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

Why are my standard curve values lower than those shown on the datasheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear, it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

How do I normalize my samples against protein concentration?

You can use a protein quantitation assay on the supernatants you get from cell/tissue lysates or with any other liquid sample in the assay buffer.

Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

18. **INTERFERENCES**

19. **NOTES**

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