For life science research only.
Not for use in diagnostic procedures.

# L-Carnitine

**Version: 22**
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Enzymatic UV test for the determination of L-Carnitine in research samples from seminal plasma, serum, or urine.

**Cat. No. 11 242 008 001** 1 test combination
3 × 10 tests

**Store the kit at +2 to +8°C.**

# 1. General Information

## 1.1. Contents

| Vial / Bottle | Label | Function / Description | Content |
|---|---|---|---|
| 1 | L-Carnitine, Coenzyme/Buffer mixture | ▪ Lyophilized<br>▪ Contains Tris buffer, pH 7.0, 5 mg NADH, 6 mg ATP, 4 mg acetyl-coenzyme A, 3 mg PEP, magnesium acetate, and stabilizers. | 3 bottles, 0.7 g each |
| 2a | L-Carnitine, Enzyme Component A | ▪ Lyophilized<br>▪ Acetyl-CoA synthetase (ACS) >2 U. | 1 bottle |
| 2b | L-Carnitine, Enzyme Blend B | Enzyme suspension containing approximately 160 U myokinase, 240 U lactate dehydrogenase, and 240 U pyruvate kinase. | 1 bottle, 3 ml |
| 3 | L-Carnitine, Carnitine acetyltransferase | Ready-to-use enzyme suspension containing approximately 60 U Carnitine acetyltransferase. | 1 bottle, 0.2 ml |
| 4 | L-Carnitine | ▪ Stabilized<br>▪ Ready-to-use L-Carnitine standard solution, approximately 100 mg/l.<br>*See bottle label for exact concentration.* | 1 bottle, 3 ml |
| 5 | L-Carnitine, Detergent | Ready-to-use solution. | 1 bottle, 3.5 ml |

## 1.2. Storage and Stability

### Storage Conditions (Product)

When stored at +2 to +8°C, the kit is stable through the expiry date printed on the label.

| Vial / Bottle | Label | Storage |
|---|---|---|
| 1 | Coenzyme/Buffer mixture | Store at +2 to +8°C. |
| 2a | Enzyme Component A | |
| 2b | Enzyme Blend B | |
| 3 | Carnitine acetyltransferase | |
| 4 | L-Carnitine | |
| 5 | Detergent | |

## 1.3. Additional Equipment and Reagent required

### For preparation of samples

- Perchloric acid, approximately 0.6 M
- Potassium carbonate solution, approximately 1.2 M (approximately 165 g $K_2CO_3$/l water)

### For assay conditions

- Glass cuvettes (1 cm path length), or
- Disposable cuvettes

### For determination of L-Carnitine in food

- Perchloric acid (1 M)
- Potassium phosphate ($K_3PO_4$ = 1.75 M)

## 1.4. Application

L-Carnitine is intended as a research tool to increase scientific knowledge about the physiological consequences of L-Carnitine deficits.

# 2. How to Use this Product

## 2.1. Before you Begin

### Sample Materials

The L-Carnitine test can be used in research samples from serum, urine, and seminal plasma.

### General Considerations

#### References values

| Sample | Value |
|---|---|
| Serum | 6.9 mg/l or 43 µM |
| Urine | 28.2 mg/day or 175 µM/day (men) |
| | 13.9 mg/day or 86 µM/day (women) |
| Seminal fluid | 40 – 100 mg/l or 250 – 620 µM |

#### Linearity

The test is linear in the range of 5.6 to 112 µM L-Carnitine in the test solution.

#### L-Carnitine standard solution

When analyzing the L-Carnitine standard solution (Bottle 4), the dilution factor F is 1.
*The standard solution contains approximately 100 mg/l; exact concentration is on the bottle label.*

### Safety Information

#### For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

#### Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis/ Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

#### Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

# Working Solution

## Preparation of kit working solutions

| Solution | Preparation | Storage and Stability |
|---|---|---|
| Solution 1 | ▪ Dissolve contents of one Bottle 1 (Coenzyme/Buffer mixture) with 10 ml double-distilled water.<br>▪ Add 1 ml from Bottle 5 (Detergent) and mix. | ▪ Store 4 days at +2 to +8°C.<br>▪ Equilibrate 10 minutes at +15 to +25°C prior to use. |
| Solution 2 | Dissolve lyophilized Enzyme Component A (ACS, Bottle 2a) with the enzyme suspension, Enzyme Blend B (Bottle 2b). | Store for 3 months at +2 to +8°C. |

# 2.2. Protocols

## Preparation of serum or plasma samples

1. Collect blood from a non-congested vein into a test tube that may contain EDTA.

2. Prepare serum or plasma according to standard methods.

### Deproteinization

*See section,* **Additional Equipment and Reagent Required** *for additional information on preparing solutions.*

1. Pipette 1 ml 0.6 M Perchloric acid solution and 1 ml ice-cooled serum or plasma sample into 10 ml centrifuge tubes.

2. Mix and keep on ice for 10 minutes.
   – Centrifuge at 3,000 × *g* (6,000 rpm, r = 7 cm) for 10 minutes.

3. Pipette 1 ml of the supernatant into a new tube.
   *Supernatant may be slightly turbid.*

4. Add 200 µl Potassium carbonate solution, approximately 1.2 M.

5. Mix and incubate on ice for 20 minutes.

6. Centrifuge at 3,000 × *g* (6,000 rpm, r = 7 cm) for 5 minutes.

7. Transfer the supernatant into a new tube.

*The separated supernatant is stable for 5 days at +2 to +8°C in a closed vial.*

### Dilution factor

The calculated dilution factor is F = 2.34.
($P_{serum}$ = 1.03 g/ml; liquid $portion_{serum}$: 0.92).

## Preparation of urine samples

Use untreated urine for the test.

*Urine samples stored at +2 to +8°C are stable for approximately 3 days.*

1. Collect a 24 hour urine sample.

2. Measure the total volume of the urine.

### Dilution factor

Urine is used undiluted; the dilution factor is F = 1.0.

## Preparation of seminal plasma samples

*See section,* **Additional Equipment and Reagent Required** *for additional information on preparing solutions.*

1. Pipette 0.5 ml 0.6 M Perchloric acid solution and 0.5 ml ice-cooled seminal plasma sample into 10 ml centrifuge tubes.

2. Mix and keep on ice for 10 minutes.
   – Centrifuge at 3,000 × *g* (6,000 rpm, r = 7 cm) for 10 minutes.

3. Pipette 500 µl of the supernatant into a new tube.
   *Supernatant may be slightly turbid.*

4. Add 100 µl Potassium carbonate solution, approximately 1.2 M.

5. Mix and incubate on ice for 20 minutes.

6. Centrifuge at 3,000 × *g* (6,000 rpm, r = 7 cm) for 5 minutes.

7. Transfer the supernatant into a new tube.

*The separated supernatant is stable for 5 days at +2 to +8°C in a closed vial.*

### Calculation of the dilution factor F

The deproteinization and neutralization procedures require a dilution factor F. The density (p = 1.035 g/ml) and the liquid portion of seminal plasma (98% = 0.98) must be taken into account in calculating this factor. If 0.5 ml seminal plasma, 0.5 ml Perchloric acid solution. 0.5 ml supernatant after deproteinization, and 0.1 ml Potassium carbonate solution are used, the dilution factor is:

$$F = \frac{(0.5 \times 1.035 \times 0.98) + 0.5}{0.5} \times \frac{0.6}{0.5} = 2.42$$

## Absorbance measurement

*See section,* **Working Solution** *for additional information on preparing solutions.*

1 Perform measurement against air without a cuvette in the light path, or against water.
*Commercially available disposable cuvettes may be used instead of glass cuvettes.*

| Parameter | Value |
|---|---|
| Wavelength | 340 nm, Hg 365 nm, or Hg 334 nm<br>*The absorption maximum of NADH is at 340 nm. With spectrophotometers, measurements are carried out at the absorption maximum.*<br>*When working with spectral-line photometers equipped with a mercury vapor lamp, measurements are carried out at 365 nm or 334 nm.* |
| Glass cuvette | 1 cm light path |
| Temperature | +15 to +25°C |
| Assay volume | 2.205 ml |

2 Pipette the following reagents into the cuvettes and mix.

| Reagent | Blank | Sample | Standard (optional) |
|---|---|---|---|
| Solution 1 | 1 ml | 1 ml | 1 ml |
| Sample (serum, plasma, or untreated urine)<br>*For deproteinized seminal plasma, use 200 µl sample volume and add 900 µl double-distilled water.* | – | 500 µl | – |
| Standard solution | – | – | 100 µl |
| Suspension 2 (Bottle 2a + 2b) | 100 µl | 100 µl | 100 µl |
| Double-distilled water | 1.1 ml | 600 µl | 1 ml |

3 Measure the absorbance of the solutions after 10 minutes ($A_1$).

4 Start the reaction by adding 5 µl Suspension 3 (Bottle 3) and mix.

5 Exactly 30 minutes after adding Suspension 3, measure the absorbance of the solutions in quick succession ($A_2$).

6 Measure the absorbance again exactly 10 minutes later ($A_3$).

### Absorbance difference

- Absorbance difference of the blank = $(A_1 - A_2)_{blank} - 3 \times (A_2 - A_3)_{blank} = (\Delta A_{blank})$
- Absorbance difference of the sample = $(A_1 - A_2)_{sample} - 3 \times (A_2 - A_3)_{sample} = (\Delta A_{sample})$

Subtract the absorbance difference of the blank from the absorbance difference of the sample to obtain ΔA.

*Dilute the sample if $\Delta A_{sample}$ is higher than 1.100 or 0.600, respectively measured at 340 nm, Hg 334 nm, or Hg 365 nm.*

Because of the high L-Carnitine concentration in seminal plasma, it is usually not necessary to increase the sample volume.

## Calculation

According to the general equation for reactions in which the amount of NADH consumed is equivalent to half the amount of substrate, the concentration is calculated by:

$$c = \frac{V \times MW \times F}{\epsilon \times d \times v \times 2} \times \Delta A \text{ (mg/l)}$$

V = final volume (ml)
v = sample volume (ml)
MW = molecular weight of the substance to be assayed (g/mol)
d = path length (cm)
ε = absorbance coefficient of NADH at 340 nm = 6.3 (1 × $mmol^{-1}$ × $cm^{-1}$)

- Hg 365 nm = 3.4 (1 × $mmol^{-1}$ × $cm^{-1}$)
- Hg 334 nm = 6.18 (1 × $mmol^{-1}$ × $cm^{-1}$)

F = dilution factor
For L-Carnitine, the concentration is:

$$c = \frac{2.205 \times 161.2 \times F}{\epsilon \times 1 \times 0.1 \times 2} \times \Delta A$$

$$= \frac{1777 \times F}{\epsilon} \times \Delta A$$

$$= \text{(mg L-carnitine/l sample solution)}$$

### For deproteinized seminal plasma

$$c = \frac{2.205 \times 161.2 \times 2.42}{\epsilon \times 1 \times 0.2 \times 2} \times \Delta A$$

$$= \frac{2150}{\epsilon} \times \Delta A$$

$$= \text{(mg L-carnitine/l sample solution)}$$

### For urine

$$c = \frac{2.205 \times 161.2 \times 1}{\epsilon \times 1 \times 0.5 \times 2} \times \Delta A$$

$$= \frac{355.4}{\epsilon} \times \Delta A$$

$$= \text{(mg L-carnitine/l sample solution)}$$

### For serum or plasma

$$c = \frac{2.205 \times 161.2 \times 2.34}{\epsilon \times 1 \times 0.5 \times 2} \times \Delta A$$

$$= \frac{831.7}{\epsilon} \times \Delta A$$

$$= \text{(mg L-carnitine/l sample solution)}$$

# Determination of L-Carnitine in food

## Sample preparation

*See section,* **Additional Equipment and Reagent Required** *for additional information on preparing solutions.*

1. Accurately weigh approximately 1 g of the homogenized sample into a 100 ml beaker and add 50 ml double-distilled water.
   *For low L-Carnitine contents, increase up to 5 g weight of sample.*
2. Stir the mixture for approximately 5 minutes.
3. Add 5 ml 1 M Perchloric acid and stir for an additional 5 minutes.
4. Adjust the pH to 7.5 with approximately 3 ml Potassium phosphate ($K_3PO_4$ = 1.75 M).
5. Transfer the mixture quantitatively into a 100 ml flask and fill up to the mark with double-distilled water.
6. Mix and filter.
7. Discard the first few ml, then remove an aliquot of approximately 10 ml; cool on ice for 5 to 10 minutes.
8. Centrifuge at 8,000 × *g* (10,000 rpm, r = 7 cm) for 5 minutes.
9. Use 100 µl of the supernatant for the assay.

*For low L-Carnitine contents, increase the sample volume up to 500 µl and reduce the water content.*

### Calculation of the concentration

For L-Carnitine, the concentration in food is:

$$c = \frac{2.205 \times 161.2}{\epsilon \times 1.0 \times 0.1 \times 2} \times \Delta A$$

$$= \frac{1777}{\epsilon} \times \Delta A$$

$$= (\text{mg L-carnitine/l sample solution})$$

If the sample has been diluted during sample preparation, the result must be multiplied by the dilution factor F.

$$\text{Cont. L-carnitine} = \frac{c_{\text{L-carnitine}}\ (\text{g/l sample sol.}) \times 100\ (\text{g/100 g})}{\text{weight}_{\text{sample}}\ (\text{g/sample sol.})}$$

### Dilution table

| Estimated amount of L-Carnitine/ liter measurements [g] | Dilution with water | Dilution factor F |
|---|---|---|
| <0.180 | – | 1 |
| 0.180 – 1.8 | 1 + 9 | 10 |
| 1.8 – 18 | 1 + 99 | 100 |
| >18 | 1 + 999 | 1,000 |

# 3. Additional Information on this Product

## 3.1. Test Principle

### How this product works

1. L-Carnitine is acetylated to acetyl carnitine by acetyl-coenzyme A (acetyl-CoA) in the presence of the enzyme carnitine acetyltransferase (CAT).

$$\text{L-carnitine} + \text{acetyl CoA} \xrightarrow{\text{CAT}} \text{acetyl carnitine} + \text{CoA}$$

2. The resulting coenzyme A (CoA) is acetylated back to acetyl-CoA in the presence of adenosine-5′-triphosphate (ATP) and acetate, catalyzed by the enzyme acetyl-CoA synthetase (ACS).
   – This results in the formation of adenosine-5′-monophosphate (AMP) and inorganic pyrophosphate ($PP_i$).

$$\text{CoA} + \text{ATP} + \text{acetate} \xrightarrow{\text{ACS}} \text{acetyl CoA} + \text{AMP} + PP_i$$

3. In the presence of ATP, supported by myokinase (MK), AMP forms twice the amount of adenosine-5′-diphosphate (ADP).

$$\text{AMP} + \text{ATP} \xrightarrow{\text{MK}} 2\ \text{ADP}$$

4. This is converted in the following reaction with phosphoenolpyruvate (PEP) in the presence of pyruvate kinase (PK).

$$2\ \text{ADP} + 2\ \text{PEP} \xrightarrow{\text{PK}} 2\ \text{ATP} + 2\ \text{pyruvate}$$

5. The pyruvate formed is reduced to L-lactate by reduced nicotinamide adenine dinucleotide (NADH) in the presence of lactate dehydrogenase (LDH).

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$$2\ \text{pyruvate} + 2\ \text{NADH} + 2\ H^+ \xrightarrow{\text{LDH}} 2\ \text{L-lactate} + 2\ NAD^+$$

The amount of NADH consumed during the reaction is equivalent to half the amount of L-Carnitine. NADH is the parameter to be measured. It is determined on the basis of its absorption at 334 (Hg), 340, or 365 (Hg) nm.

# 4. Supplementary Information

## 4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

| Text convention and symbols | |
|---|---|
| *Information Note: Additional information about the current topic or procedure.* | |
| **Important Note: Information critical to the success of the current procedure or use of the product.** | |
| ① ② ③ etc. | Stages in a process that usually occur in the order listed. |
| ❶ ❷ ❸ etc. | Steps in a procedure that must be performed in the order listed. |
| * (Asterisk) | The Asterisk denotes a product available from Roche Diagnostics. |

## 4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

## 4.3. Trademarks

All product names and trademarks are the property of their respective owners.

## 4.4. License Disclaimer

For patent license limitations for individual products please refer to:
**List of biochemical reagent products**.

## 4.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

## 4.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

## 4.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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