L-Carnitine

Enzymatic UV test for the determination of L-carnitine in research samples from seminal plasma, serum or urine.

**Cat. No. 11 242 008 001**

Test-Combination for approx. 3 × 10 determinations

1. **Product overview**

**Kit Contents**

<table>
<thead>
<tr>
<th>Bottle</th>
<th>Contents</th>
</tr>
</thead>
</table>
| 1      | • 3 × 0.7 g coenzyme buffer/mixture.  
         • lyophilisate  
         • consisting of  
         • Tris buffer, pH 7.0;  
         • NADH, 5 mg;  
         • ATP, 6 mg;  
         • Acetyl-coenzyme A, 4 mg;  
         • PEP, 3 mg;  
         • Magnesium acetate and stabilizers. |
| 2a     | Enzyme Component A:  
         • 3 ml enzyme suspension  
         • consisting of  
         • myokinase approx. 160 U;  
         • lactate dehydrogenase approx. 240 U;  
         • pyruvate kinase approx. 240 U. |
| 2b     | Enzyme Blend B:  
         • 3 ml enzyme suspension  
         • consisting of  
         • myokinase approx. 160 U;  
         • lactate dehydrogenase approx. 240 U;  
         • pyruvate kinase approx. 240 U. |
| 3      | • 0.2 ml enzyme suspension carnitine acetyl transferase.  
         • approx. 80 U.  
         • Ready-to-use. |
| 4      | • L-carnitine standard solution.  
         • approx. 100 mg/l (exact concentration see bottle label).  
         • Ready-to-use. |
| 5      | • Detergent solution.  
         • Ready-to-use. |

**Principle (1)**

L-carnitine is acetylated to acetyl carnitine by acetyl coenzyme A (acetyl-CoA) in the presence of the enzyme carnitine acetyl transferase (CAT). 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’-diphosphate (ADP). This is converted in the following reaction with phosphoenol pyruvate (PEP) in the presence of pyruvate kinase (PK). The pyruvate formed is reduced to L-lactate by reduced nicotinamide adenine dinucleotide (NADH) in the presence of lactate dehydrogenase (LDH).

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.

1. L-carnitine + acetyl-CoA $\xrightarrow{\text{CAT}}$ acetyl carnitine + CoA
2. CoA + ATP + acetate $\xrightarrow{\text{ACS}}$ acetyl-CoA + AMP + PPi
3. AMP + ATP $\xrightarrow{\text{MK}}$ 2 ADP
4. 2 ADP + 2 PEP $\xrightarrow{\text{PK}}$ 2 ATP + 2 pyruvate
5. 2 pyruvate + 2 NADH + 2 H$^+$ $\xrightarrow{\text{LDH}}$ 2 L-lactate + 2 NAD$^+$

**2. Procedures and required material**

2.1 **Before you begin**

**Preparation of kit working solutions**

- To prepare solution 1, dissolve contents of one bottle with 10 ml double dist. water. Add 1 ml from bottle 5 (detergent) and mix.
- Before use keep solution 10 min at +15 to +25°C.
- To prepare solution 2, dissolve ACS lyophilisate from bottle 2a with the enzyme suspension (bottle 2b).

**Standard solution**

When analyzing the standard solution contained in the Test-Combination, the dilution factor F is 1. The standard solution contains approx. 100 mg/l (exact concentration see bottle label).

2.2 **Preparation of serum or plasma samples**

**Additional reagents required**

- Perchloric acid, approx. 0.6 M.
- Potassium carbonate solution, approx. 1.2 M (approx. 165 g K$_2$CO$_3$/l water).

**Procedure**

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Remove blood from an uncongested vein and fill into a test tube which may contain EDTA.</td>
</tr>
<tr>
<td>2</td>
<td>Prepare serum or plasma in the usual way.</td>
</tr>
</tbody>
</table>
Deproteinization

Serum or plasma has to be deproteinized as follows.

### Dilution factor

The calculated dilution factor is \( F = 2.34 \).

\[
\rho_{\text{serum}} = 1.03 \text{ g/ml; liquid portion serum: 0.92}.
\]

2.3 Preparation of urin samples

**Protocol**

Use untreated urine for the test.

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

**Dilution factor**

Urine is used undiluted, the dilution factor is \( F = 1.0 \).

2.4 Preparation of seminal plasma samples

**Additional required reagents**

- Perchloric acid, approx. 0.6 M.
- Potassium carbonate solution, approx. 1.2 M (approx. 165 g K₂CO₃/l water).

**Protocol**

1. Pipet into 10-ml centrifuge tubes:
   - 1 ml Perchloric acid solution (0.6 M).
   - 1 ml Ice-cooled seminal plasma.
2. Mix.
3. Pipet 1 ml of the supernatant into a new tube.
4. Add 200 µl Potassium carbonate solution (approx. 1.2 M).
5. Mix and incubate on ice for 20 min.
6. Centrifuge at 3000 \( \times \) g (6000 rpm, \( r = 7 \) cm) for 5 min.
7. Transfer the supernatant into a new tube.

**Calculation of the dilution factor \( F \)**

The deproteinization and neutralization procedures require a dilution factor \( F \). The density \( \rho = 1.035 \text{ g/ml} \) and the liquid portion of seminal plasma \( (98\% = 0.98) \) have to 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:

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

3. Standard protocol

**Additional equipment required**

Glass cuvette: 1 cm path length.

**Wavelength**

340 nm, Hg 365 nm or Hg 334 nm.

**Protocol**

Measure against air (without a cuvette in the light path) or against water.

**Temperature:** +15 to +25°C

**Final volume:** 2.205 ml

**Absorbance difference**

Absorbance difference of the blank

\[
(\Delta A)_{\text{blank}} = \frac{A_1 - A_2}{3} \times \frac{A_2 - A_3}{\Delta A_{\text{blank}}}
\]

Absorbance difference of the sample

\[
(\Delta A)_{\text{sample}} = \frac{A_1 - A_2}{3} \times \frac{A_2 - A_3}{\Delta A_{\text{sample}}}
\]

Subtract the absorbance difference of the blank from the absorbance difference of the sample to obtain \( \Delta A \).

**Note:** Dilute the sample if \( \Delta A_{\text{sample}} \) 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.
4. Calculation

General

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:

\[ c = \frac{V \times MW \times F}{\varepsilon \times d \times v \times 2} \times \frac{\Delta A}{Hg} \] (mg/l)

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

For L-carnitine the concentration is:

\[ c = \frac{2.205 \times 1612 \times F}{\varepsilon \times 1 \times 0.1 \times 2} \times \frac{\Delta A}{Hg} \] (mg L-carnitine/l sample solution)

Dilution table

<table>
<thead>
<tr>
<th>Estimated amount of L-carnitine per liter measurements</th>
<th>Dilution with water</th>
<th>Dilution factor F</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.180 g</td>
<td>1 + 9</td>
<td>100</td>
</tr>
<tr>
<td>0.180 - 1.8 g</td>
<td>1 + 99</td>
<td>100</td>
</tr>
<tr>
<td>1.8 - 18 g</td>
<td>1 + 999</td>
<td>1000</td>
</tr>
</tbody>
</table>

5. Determination of L-carnitine in food

Sample preparation

Please refer to the following table.

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Accurately weigh approx. 1 g of the homogenized sample into a 100 ml beaker and add 50 ml double distilled water. <strong>Note:</strong> In case of low L-carnitine contents increase up to 5 g weight</td>
</tr>
<tr>
<td>2</td>
<td>Stir the mixture for approx. 5 min</td>
</tr>
<tr>
<td>3</td>
<td>Add 5 ml perchloric acid (1 M) and stir for another 5 min.</td>
</tr>
<tr>
<td>4</td>
<td>Adjust pH to 7.5 with approx. 3 ml potassium phosphate (K2HPO4 = 1.75 M)</td>
</tr>
<tr>
<td>5</td>
<td>Transfer the mixture quantitatively into a 100-ml flask and fill up to the mark with double distilled water.</td>
</tr>
<tr>
<td>6</td>
<td>Mix and filter.</td>
</tr>
<tr>
<td>7</td>
<td><strong>Note:</strong> In case of low L-carnitine contents, increase the sample volume up to 500 µl and reduce the water content.</td>
</tr>
<tr>
<td>8</td>
<td>Centrifuge for 5 min at 8000 x g (10.000 rpm, r = 7 cm).</td>
</tr>
<tr>
<td>9</td>
<td>Use 100 µl of the supernatant for the assay. <strong>Note:</strong> In case of low L-carnitine contents, increase the sample volume up to 500 µl and reduce the water content.</td>
</tr>
</tbody>
</table>

6. References


Changes to Previous Version

- Editorial changes
- Regulatory Disclaimer updated

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