L-Citrulline Kit

For the in vitro determination of L-Citrulline in urine, serum and plasma

Gültig ab / Valid from 26.10.2012

REF K6600
1. INTENDED USE

The described Assay is intended for the quantitative determination of L-Citrulline in urine, serum and plasma. For in vitro diagnostic use only.

2. INTRODUCTION

Nitric oxide (NO) is an intra- and intercellular signaling molecule. It reacts with free radicals, metalloproteins and specific amino acid residues of proteins. NO plays an important role in the regulation of vascular tone. Endothelial NO (eNO) is produced by the vascular endothelium. It diffuses to neighboring vascular smooth muscle cells (VSMC), where NO activates soluble guanylate cyclase (sGC), which subsequently increases the intracellular cGMP production from GTP, and which in turn causes relaxation of smooth muscle and vasodilatation.

Thus, functional changes of the endothelium in coronary artery disease may be an important factor in the development of vasospasm, ischaemia and thrombosis.

L-citrulline as surrogate marker for NO.

NO is synthesized in the citrulline-NO-cycle when L-arginine is oxidized to citrulline by NO synthase (NOS). In the second part of the urea cycle, arginine is re-synthesized from citrulline. The NOS catalyzed formation of L-citrulline and NO proceeds in two steps, whereby the product stochiometry of L-citrulline and NO is 1:1. Thus, the conversion of L-arginine to L-citrulline can be used as a surrogate marker for the NO synthesis.

Pathologic high levels of citrulline serve as an indicator of nitrosative stress.

Indications

- Estimation of NOS activity (NO production)
- Detection of nitrosative stress due to an enhanced synthesis of inducible nitric oxide (iNO)
3. MATERIAL SUPPLIED

<table>
<thead>
<tr>
<th>Catalogue No</th>
<th>Content</th>
<th>Kit Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K6600F</td>
<td>PREC</td>
<td>Precipitation regent</td>
<td>1 x 10 ml</td>
</tr>
<tr>
<td>K6600ST</td>
<td>STD</td>
<td>Standard concentrate (40 mM/L L-Citrulline)</td>
<td>1 x 50 μl</td>
</tr>
<tr>
<td>K6600VP</td>
<td>STDBUF</td>
<td>Standard dilution buffer</td>
<td>1 x 20 ml</td>
</tr>
<tr>
<td>K6600LA</td>
<td>SOL A</td>
<td>Solution A</td>
<td>1 x 10 ml</td>
</tr>
<tr>
<td>K6600LB</td>
<td>SOL B</td>
<td>Solution B</td>
<td>1 x 40 ml</td>
</tr>
<tr>
<td>K6600MTP</td>
<td>PLATE</td>
<td>Microtiter plate</td>
<td>2 x</td>
</tr>
<tr>
<td>K6600PVP</td>
<td>SAMDIL</td>
<td>Sample dilution buffer, lyophilized</td>
<td>4 x 2 ml</td>
</tr>
<tr>
<td>K6600KO1</td>
<td>CTRL</td>
<td>Control, ready to use</td>
<td>4 x 200 μl</td>
</tr>
<tr>
<td>K6600KO2</td>
<td>CTRL</td>
<td>Control, ready to use</td>
<td>4 x 200 μl</td>
</tr>
</tbody>
</table>

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Precision pipettors and disposable tips to deliver 10-1000 μl
- Multi-channel dispenser or repeating dispenser
- Centrifuge capable of min. 3000 x g
- Vortex-Mixer
- Heated incubator at 90°C
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 540 nm
- Water bath at 37°C
- Metal frame for the microtiter plate modules

*Immundiagnostik AG recommends the use of Ultra Pure Water (Water Type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 μm) with an electrical conductivity of 0.055 μS/cm at 25°C (≤18.2 MΩ cm).
5. PREPARATION AND STORAGE OF REAGENTS

- Reconstitute **SAMDIL** (sample dilution buffer) with 2 ml of ultra pure water, mix gently, allow the vial content to dissolve for **5 minutes** at room temperature and mix again.

- **Standard curve preparation**

  Prepare from the L-Citrulline stock solution (**STD**) a standard curve according to the following scheme:

  **Standard 1** (400 μM/L): dilute L-Citrulline stock solution (STD) (40 mM/L) 1:100 with standard dilution buffer (STDBUF)

  **Standard 2** (200 μM/L): Standard 1 1:2 diluted with STDBUF

  **Standard 3** (100 μM/L): Standard 2 1:2 diluted with STDBUF

  **Standard 4** (50 μM/L): Standard 3 1:2 diluted with STDBUF

  **Standard 5** (25 μM/L): Standard 4 1:2 diluted with STDBUF

  **Standard 6** (12.5 μM/L): Standard 5 1:2 diluted with STDBUF

  **Standard 7** (6.25 μM/L): Standard 6 1:2 diluted with STDBUF

  For **Standard 8** a standard dilution buffer is used.

- **Preparation of the color solution**

  Mix 1 part solution A (SOL A) with 3 parts solution B (SOL B). (Prepare fresh color solution for each assay, because it is stable only for around 30 minutes). Store SOL A and SOL B at 2-8°C and bring at room temperature before use.

- **STD** (L-Citrulline stock solution), **CTRL** (controls) and **SAMDIL** (sample dilution buffer) should be stored at -20°C before use.

- **STD** (L-Citrulline stock solution) can be freeze-thawed up to four times.

- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at **2-8°C**.
6. SAMPLE PREPARATION

- Pipette 500 μl of sample in 1.5 ml reaction vial
- Add 100 μl of reconstituted sample buffer (SAMDIL) to the sample
- Mix well
- Incubate for 1 h at 37°C
- Add 150 μl of cold (2-8°C) precipitation reagent (PREC)
- Mix well
- Incubate for 30 min at 2-8°C
- Centrifuge at 3,000 x g for 10 min
- Use the supernatant in the test

7. ASSAY PROCEDURE

Principle of the test

After a sample pre-treating to eliminate the interference of other substances, a development solution composed of two components is added to the sample. The color changes to intensive red due to the reaction of L-Citrulline with DAMO. The interference of reaction byproducts is reduced by TSC-treating.

The color intensity is proportional to the analyte concentration. The absorbance is measured at 540 nm. The concentration of the samples is estimated using a standard curve. In order to eliminate the effect of the sample matrix on the absorption, an individual sample blank should be run. The obtained blank value is subtracted from the sample result.
**Test procedure**

1. Bring all **reagents and samples** to **room temperature** (18-26 °C) and mix well.

2. Mark the positions of **STD/CTRL/SAMPLE** (Standard/Control/Sample) in duplicate on a protocol sheet.

3. Loosen the strips of the MTP, so that they can be easily taken out.
   Pipette **2 x 60 µl STD/CTRL** (Standard/Control) in the microtiter plate (MTP) (2 wells per standard/control; 60 µl in each).

4. Pipette **4 x 60 µl of the prepared sample** in the MTP (4 wells per sample; 60 µl in each).

5. Add **200 µl of color solution** in all standard-wells and in 2 of the sample-wells.

6. Add **200 µl of SOL B** in the 2 remaining sample-wells (these without color solution) (sample blank).

7. **Cover microtiter plate strips**, take them out and bring them in a **metal holder pre-heated top 90°C**.

8. **Incubate at 90°C for 15 minutes**.

9. Take the **microtiter plate strips out of the heater** and put in the original holder.

10. **Let cool down to room temperature for 10 minutes** (The samples are stable for app. 30 minutes).

11. Read absorption with an ELISA – Reader **at 540 nm**.
8. RESULTS

The final L-Citrulline concentration in μmol/L is calculated as a difference between the sample concentration with the color solution and the concentration of the sample blank (sample with SOL B) multiplied by 1.5.

\[
\text{L-Citrulline [μmol/l]} = ([\text{measured content}] - [\text{measured content of the blank}]) \times 1.5
\]

9. QUALITY CONTROL

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

**Expected values**

**Normal ranges**

We recommend each laboratory to establish its own norm concentration range.

10. PERFORMANCE CHARACTERISTICS

**Precision and reproducibility**

<table>
<thead>
<tr>
<th>Intra-Assay (n=12)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Citrulline [μmol/l]</td>
<td>Standard variation (SD) [%]</td>
</tr>
<tr>
<td>1</td>
<td>32.9</td>
<td>1.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-Assay (n=7)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Citrulline [μmol/l]</td>
<td>Standard variation (SD) [%]</td>
</tr>
<tr>
<td>1</td>
<td>54.7</td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>48.9</td>
<td>3.7</td>
</tr>
</tbody>
</table>
Sensitivity

The sensitivity was set as $B_0 + 2SD$. The zero-standard was measured 20 times.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Citrulline mean value [OD]</th>
<th>2 x Standard variation (2 x SD) [%]</th>
<th>Detection limit [μmol/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.1</td>
<td>0.2</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Linearity

Linearity of the test was determined by diluting a patient sample.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Measured [μmol/l]</th>
<th>Expected [μmol/l]</th>
<th>Recovery [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>original</td>
<td>100</td>
<td>100</td>
<td>100.0</td>
</tr>
<tr>
<td>1 zu 1.2</td>
<td>87.4</td>
<td>83.3</td>
<td>104.9</td>
</tr>
<tr>
<td>1 zu 1.5</td>
<td>71.2</td>
<td>66.6</td>
<td>106.9</td>
</tr>
<tr>
<td>1 zu 3.0</td>
<td>37.2</td>
<td>33.3</td>
<td>111.7</td>
</tr>
</tbody>
</table>

11. PRECAUTIONS

- For *in vitro* diagnostic use only.
- Quality control guidelines should be followed.
- Solution B (SOL B) contains a strong acid and must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water.
12. TECHNICAL HINTS

- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown on the kit label.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- The assay should always be performed according the enclosed manual.

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for in vitro diagnostic use only.
- Guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

14. REFERENCES

