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Manual

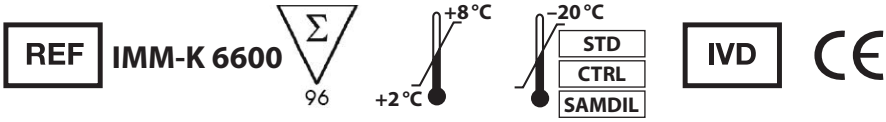
L-Citrulline Kit

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

EU: IVD / CE

US: Research Use Only. Not for use in diagnostic procedures.

Valid from 14.04.2014



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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

Cat. No.	Label	Kit components	Quantity
K 6600F	PREC	Precipitation regent	1 x 10 ml
K 6600ST	STD	Standard concentrate (40 mM/l L-citrulline)	1 x 50 µl
K 6600VP	STDBUF	Standard dilution buffer	1 x 20 ml
K 6600LA	SOL A	Solution A	1 x 10 ml

Cat. No.	Label	Kit components	Quantity
K 6600LB	SOL B	Solution B	1 x 40 ml
K 6600MTP	PLATE	Microtiter plate	2 x
K 6600PVP	SAMDIL	Sample dilution buffer, lyophilized	4 x 2 ml
K 6600KO1	CTRL	Control, ready to use	4 x 200 µl
K 6600KO2	CTRL	Control, ready to use	4 x 200 µl

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Calibrated precision pipettors and 10–1000 µl tips
- Foil to cover the microtiter plate
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 *g*
- Heated incubator at 90 °C
- Water bath at 37 °C
- Metal frame for the microtiter plate modules
- Vortex
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 540 nm

* 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

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.**
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- Reconstitute lyophilized **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. **Reconstituted SAMDIL cannot be stored.**
- **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** 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 it to room temperature before use.
- **STD** (L-Citrulline stock solution), **CTRL** (controls) and **SAMDIL** (sample dilution buffer) should be stored at **-20 °C** before use. They 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. SPECIMEN COLLECTION AND PREPARATION

Note

Standards and samples should be pipetted without air bubbles.

1.	Pipet **500 µl of sample** in 1.5 ml reaction vial
2.	Add **100 µl of reconstituted sample buffer** (SAMDIL) to the sample
3.	Mix well
4.	Incubate for **1 h at 37 °C**
5.	Add **150 µl** of **cold (2–8 °C) precipitation reagent** (PREC)
6.	Mix well
7.	Incubate for **30 min at 2–8 °C**
8.	**Centrifuge** at 3.000 *g* for **10 min**
9.	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

We recommend to carry out the tests in duplicate.

It is recommended to switch on the oven at 90 °C and place the metal frame for the microtiter plate modules in it before the start of the test procedure.

1.	Bring **all reagents and samples** to **room temperature** (15–30 °C) and mix well
2.	Mark the positions of **STD/CTRL/SAMPLE** (standards/controls/samples) on a protocol sheet
3.	Loosen the strips of the MTP, so that they can be easily taken out. Pipet **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 to 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 ~ 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.

L-citrulline [µmol/l] = ([measured content$_{sample}$] – [measured content$_{blank}$]) * 1.5

9. LIMITATIONS

Samples with an OD higher than the OD of the highest standard should be further diluted and re-assayed. For the following analysis, the changed dilution factor has to be taken into consideration.

10. QUALITY CONTROL

Immundiagnostik recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed 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.

Reference range

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 12)

Sample	Citrullin [µmol/l]	Standard deviation (SD) [%]
1	32,9	1,3

Inter-Assay (n = 7)

Sample	Citrullin [µmol/l]	Standard deviation (SD) [%]
1	54,7	3,0
2	48,9	3,7

Dilution recovery

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

Dilution	Measured [µmol/l]	Expected [µmol/l]	Recovery [%]
original	100	100	100,0
1:1,2	87,4	83,3	104,9
1:1,5	71,2	66,6	106,9
1:3,0	37,2	33,3	111,7

Analytical Sensitivity

The Zero-standard was measured 20 times. The detection limit was set as B_0 + 2 SD.

Sample	Citrullin mean value [OD]	2 standard deviations (2 x SD)[%]	Detection limit [µmol/l]
Zero-standard	1,1	0,2	1,5

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Control samples should be analyzed with each run.
- 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.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay.
- Reagents should not be used beyond the expiration date stated on 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.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The 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 incorrect use.
- Warranty claims and complaints regarding 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.

15. REFERENCES

1. Knipp, M, and M Vasák. 2000. "A Colorimetric 96-Well Microtiter Plate Assay for the Determination of Enzymatically Formed Citrulline." *Analytical Biochemistry* **286** (2) (November 15): 257–64. doi:10.1006/abio.2000.4805.
2. Kuklinski, B. 2005. "Zur Praxisrelevanz von Nitrosativem Stress." *Umwelt-Medizin-Gesellschaft* **18** (2): 89–172.
3. Wanchu, A, M Khullar, K Sud, V Sakhuja, K Thennarasu, A Sud, and P Bambery. 2001. "Serum and Urine Nitrite and Citrulline Levels among Patients with Systemic Lupus Erythematosus: A Possible Addition to Activity Parameters?" *Journal of Clinical Rheumatology : Practical Reports on Rheumatic & Musculoskeletal Diseases* **7** (1) (February): 10–5; discussion 15.
4. Yu, Jin-Ju, and Suk-Heung Oh. 2010. "Isolation and Characterization of Lactic Acid Bacteria Strains with Ornithine Producing Capacity from Natural Sea Salt." *Journal of Microbiology (Seoul, Korea)* **48** (4) (August): 467–72. doi:10.1007/s12275-010-0204-9.

Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

Contains sufficient for <n> tests


Manufacturer


Use by

Lot number