Version 30-12/24

User's Manual

L-Citrulline Assay Kit

REF **DEIA-JY2422**

Σ **96T**

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

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

General Description

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 neighbouring 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 synthesised in the **citrulline-NO-cycle** when L-arginine is oxidised to citrulline by NO synthase (NOS). In the second part of the urea cycle, arginine is re-synthesised from citrulline. The NOS catalysed 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)

Principles of Testing

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 colour changes to intensive red due to the reaction of L-citrulline with DAMO. The interference of reaction byproducts is reduced by TSC-treating.

The colour 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.

Reagents And Materials Provided

1. **PREC** Precipitation reagent: 1 × 20 mL
2. **STD** Standard concentrate (40 mM/L L-citrulline) 1 × 50 µL
3. **STDBUF** Standard dilution buffer 1 × 20 mL

4. **SOL A** Solution A 1 × 10 mL
5. **SOL B** Solution B 1 × 40 mL
6. **PLATE** Microtiter plate 2 ×
7. **SAMDIL** Sample dilution buffer, lyophilised 4× 2mL
8. **CTRL1** Control 1, ready-to-use 4 × 200 µL
9. **CTRL2** Control 2, ready-to-use 4 × 200 µL

Materials Required But Not Supplied

1. Ultrapure water*
2. Calibrated precision pipettors and 10–1000 µL single-use tips
3. Foil to cover the microtiter plate
4. Multi-channel pipets or repeater pipets
5. Centrifuge, 3000 ×g
6. Heated incubator at 90°C
7. Water bath at 37°C
8. Metal frame for the microtiter plate strips
9. Vortex
10. Standard single-use laboratory glass or plastic vials, cups, etc.
11. Microtiter plate reader at 540 nm

Storage

1. L-Citrulline stock solution (STD), controls (CTRL) and sample dilution buffer (SAMDIL) should be stored at -20°C before use. They can be freezethawed up to four times.
2. All other test reagents are ready-to-use. Test reagents can be used until the expiry date (see label) when stored at 2–8°C.

Specimen Collection And Preparation

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

Reagent Preparation

1. 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.** The kit can be used up to 4 times within the expiry date stated on the label.
2. Reagents with a volume less than 100 µL should be centrifuged before use to avoid loss of volume.
3. Reconstitute lyophilised sample dilution buffer (SAMDIL) with 2 mL of ultrapure water, mix gently, allow the vial content to dissolve for 5 minutes at room temperature and mix again. Sample dilution buffer (reconstituted SAMDIL) is not stable and cannot be stored.
4. **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), e.g. **10 µL** stock solution (40 mM/L) + **990 µL** STDBUF), mix well.

• **Standard 2 (200 µM/L)** : Standard 1 1:2 diluted with STDBUF, e.g. **250 µL** standard 1 (40 mM/L) + **250 µL** STDBUF), mix well.

• **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.

5. Preparation of the colour solution

Mix **1 part solution A** (SOL A) with **3 parts solution B** (SOL B). Prepare fresh colour 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.

6. **L-Citrulline stock solution (STD), controls (CTRL)** and **sample dilution buffer (SAMDIL)** should be stored at **-20°C** before use. They can be freezethawed up to four times.
7. All other test reagents are ready-to-use. Test reagents can be used until the expiry date (see label) when stored at **2–8°C**.

Assay Procedure

Bring all reagents and samples to room temperature (15–30°C) and mix well. Mark the positions of standards/controls/samples/blank for sample on a protocol sheet.

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.

Standards, controls and samples should be pipetted without air bubbles.

1. Loosen the strips of the microtiter plate (PLATE), so that they can be easily taken out. Pipet 2× 60 µL standards/controls into the into the respective wells (2 wells per standard/control; 60 µL into each).

2. Pipette 4x 60 µL of the prepared sample into the respective wells (4 wells per sample; 60 µL into each).
3. Add 200 µL of colour solution into each well for standards/controls and into 2 of the sample wells.
4. Add 200 µL of SOL B into the 2 remaining wells for the individual sample blank (these without colour solution).
5. Cover microtiter plate strips, take them out and bring them in a metal holder pre-heated to 90°C.
6. Incubate at 90°C for 15 minutes.
7. Take the microtiter plate strips out of the heater and place them in the original holder.
8. Let the strips cool down to room temperature for 10 minutes (the samples are stable for ~ 30 minutes).
9. Read absorption with an microtiter plate reader at 540 nm.

Quality Control

CD 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 statisticalmethods. 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.

Calculation

The final L-citrulline concentration in µmol/l is calculated as a difference between the sample concentration with the colour 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

Precision

Intra-Assay (n = 12): SD 1.3%

Inter-Assay (n = 7): SD 3.7%

Detection Limit

1.5 µmol/L The Zero-standard was measured 20 times. The detection limit was set as B0 + 2 SD.

Specificity

NA

Precautions

1. All reagents in the kit package are for in vitro diagnostic use only.
2. 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 up immediately with copious quantities of water.

3. Do not interchange different lot numbers of any kit component within the same assay.
4. Control samples should be analysed with each run.
5. Reagents should not be used beyond the expiration date stated on kit label.
6. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
7. Avoid foaming when mixing reagents.
8. Do not mix plugs and caps from different reagents.
9. The assay should always be performed according to the enclosed manual.

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.