Version 12-04/24

User's Manual

Citrulline ELISA Kit

REF **DEIASL211**

Σ **96T**

RUO

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

Enzyme Immunoassay for the quantitative determination of Citrulline in urine, serum, plasma and various biological samples.

General Description

Citrulline is a non-essential amino acid. In hepatocytes, L-citrulline is synthesized in the urea cycle by the addition of carbon dioxide and ammonia to ornithine. L-citrulline is converted into L-arginine by the enzymes argininosuccinate synthetase and argininosuccinate lyase in the presence of L-aspartate and ATP. Subsequently, L-arginine is converted to nitric oxide by nitric oxide synthase and L-citrulline is regenerated as a by-product.

Principles of Testing

The samples are first cleaned up by an extraction procedure. After derivatization Citrulline is quantitatively determined by ELISA. The competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The derivatized standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards.

Reagents And Materials Provided

1. Adhesive Foil: 1 x 4 foils. Ready to use.
2. Macrotiter Plate: 2 x 48 well plate, empty in a resealable pouch. Ready to use.
3. Extraction Plate: 2 x 48 well plate, precoated with cation exchanger in a resealable pouch. Ready to use.
4. Wash Buffer Concentrate (50x): Buffer with a non-ionic detergent and physiological pH, 1 x 20 mL/vial, light purple cap.
5. Enzyme Conjugate: Goat anti-rabbit immunoglobulins conjugated with peroxidase, 1 x 12 mL/vial, red cap. Ready to use.
6. Substrate: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide, 1 x 12 mL/black vial, black cap. Ready to use.
7. Stop Solution: 0.25 M sulfuric acid, 1 x 12 mL/vial, light grey cap. Ready to use.
8. Citrulline Microtiter Strips: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable pouch with desiccant. Ready to use.
9. Citrulline Antiserum: Rabbit anti-Citrulline antibody, blue coloured, 1 x 6 mL/vial, blue cap. Ready to use.
10. Assay Buffer: Buffer with alkaline pH, 1 x 20 mL/vial, yellow cap. Ready to use.
11. Equalizing Reagent Lyophilized: 1 vial, brown cap.

12. D-Reagent: Crosslinking agent in dimethylsulfoxide, 1 x 4 mL/vial, white cap. Ready to use.
13. Q-Buffer: Tris containing buffer, 1 x 20 mL/vial, white cap.
14. Diluent: Buffer with acidic pH, 2 x 20 mL/vial, dark green cap. Ready to use.
15. NaOH: Sodium hydroxide solution, red coloured, 1 x 2 mL/vial, purple cap. Ready to use.
16. Standards and Controls: Ready to use

Component	Concentration µg/mL	Concentration µmol/L	Volume/Vial
Standard A	0	0	4 mL
Standard B	0.6	3.4	4 mL
Standard C	2	11.4	4 mL
Standard D	6	34.3	4 mL
Standard E	20	114	4 mL
Standard F	60	343	4 mL
Control 1	Refer to QC report for expected value and acceptable range		4 mL
Control 2			4 mL

Conversion: Citrulline (µg/ml) x 5.71 = Citrulline (µmol/l)

Content: Acidic buffer with non-mercury preservative, spiked with defined quantity of Citrulline

Materials Required But Not Supplied

1. Calibrated precision pipettes to dispense volumes between 20 µl – 300 µl; 10 ml
2. Microtiter plate washing device (manual, semi-automated or automated)
3. ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
4. Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
5. Absorbent material (paper towel)
6. Water (deionized, distilled, or ultra-pure)
7. Vortex mixer

Storage

Store the unopened reagents at 2 - 8 C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

Specimen Collection And Preparation

Urine

Spontaneous urine or 24-hour urine, collected in a bottle containing 10 - 15 ml of 6 M HCl, can be used.

If 24-hour urine is used please record the total volume of the collected urine.

Storage: for longer periods (up to 6 month) at -20 °C.

Repeated freezing and thawing should be avoided. Avoid exposure to direct sunlight.

Plasma

Whole blood should be collected into centrifuge tubes (MonovetteTM or VacuetteTM) containing an anticoagulant and centrifuged according to manufacturer's instructions immediately after collection.

Haemolytic and lipemic samples should not be used for the assay.

Storage: for a longer period (up to 6 month) at -20 °C.

Repeated freezing and thawing should be avoided.

Serum

Collect blood by venipuncture (MonovetteTM or VacuetteTM for serum), allow to clot, and separate serum by centrifugation according to manufacturer's instructions. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Haemolytic and lipemic samples should not be used for the assay.

Storage: for a longer period (up to 6 month) at -20 °C.

Repeated freezing and thawing should be avoided.

Sample Dilution

Serum and plasma samples

Serum and plasma samples have to be diluted 1:4 with Standard A prior to the test, for example:

50 µl sample + 150 µl Standard A.

Other sample types

The Citrulline ELISA is a flexible test system for various biological sample types. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to adapt the protocol to his specific needs:

(1) It is advisable to perform a Proof of Principle to determine the recovery of Citrulline from the samples. Prepare a stock solution of Citrulline. Add small amounts (to change the native sample matrix as less as possible) of the stock solutions to the sample matrix and check the recovery.

(2) Avoid excess of acid: excess of acid might exceed the buffer capacity of the dilution buffer. A pH of 5.0 during the extraction is mandatory.

(3) Samples with concentrations above the measuring range can be diluted with the included diluent.

(4) Samples with an interfering sample matrix can also be diluted with the included diluent.

Reagent Preparation

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.

Storage: 1 month at 2 - 8 °C

Equalizing Reagent

Reconstitute the Equalizing Reagent with 10 ml of Assay Buffer.

Reconstituted Equalizing Reagent which is not used immediately has to be stored in aliquots for max 1 month

at -20 °C and may be thawed only once.

D-Reagent

The D-Reagent has a freezing point of 18.5 °C. To ensure that the D-Reagent is liquid when being used, it must be ensured that the D-Reagent has reached room temperature and forms a homogeneous, crystal-free solution.

Citrulline Microtiter Strips

In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

Assay Procedure

Assay Notes:

Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Number the extraction plate and microwell plate (microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended.

The binding of the antibodies, the enzyme conjugates, and the activity of the enzyme used are temperature dependent, the absorption values may vary if a thermostat is not used. The higher the temperature, the higher the absorption values will be. The absorption values also depend on the incubation times. The optimal temperature for the Enzyme Immunoassay is between 20 – 25 °C.

Extraction

1. Pipette 50 µl of standards, controls, urines and diluted serum and plasma samples into the respective wells of the Extraction Plate.
2. Add 300 µl of Diluent to all wells and shake for 10 min at RT (20 - 25 °C) on a shaker (approx. 600 rpm).
3. Take 100 µl of the supernatant for the derivatization.

Derivatization

1. Pipette 100 µl of the standards, controls and samples into the appropriate wells of the Macrotiter Plate.
2. Add 20 µl of the NaOH to all wells and mix shortly.
3. Add 100 µl of the Equalizing Reagent to all wells.
4. Add 20 µl of the D-Reagent to all wells.
5. Cover plate with Adhesive Foil and shake for 2 h at RT (20 - 25 °C) on a shaker (approx. 600 rpm).
6. Pipette 150 µl Q-Buffer into all wells and shake for 10 min at RT (20 - 25 °C) on a shaker (approx. 600 rpm).
7. Take 50 µl for the ELISA!

Citrulline ELISA

1. Pipette 50 µl of the prepared standards, controls and samples into the appropriate wells of the Citrulline Microtiter Strips.
2. Pipette 50 µl of the Citrulline Antiserum into all wells and mix shortly.
3. Cover plate with Adhesive Foil and incubate for 15 - 20 h (overnight) at 2 - 8 °C.
4. Remove the foil. Discard or aspirate the contents of the wells. Wash the plate 3 x by adding 300 µl of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.

5. Pipette 100 µl of the Enzyme Conjugate into all wells.
6. Incubate for 30 min at RT (20 - 25 °C) on a shaker (approx. 600 rpm).
7. Discard or aspirate the contents of the wells. Wash the plate 3 x by adding 300 µl of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
8. Pipette 100 µl of the Substrate into all wells and incubate for 20-30 min at RT (20 - 25 °C) on a shaker (approx. 600 rpm). Avoid exposure to direct sunlight!
9. Add 100 µl of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm (if available a reference wavelength between 620 nm and 650 nm is recommended).

Quality Control

The confidence limits of the kit controls are printed on the QC-Report.

Calculation

The standard curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis). Use non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

Note: This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.

The concentrations of the controls and urine samples can be read directly from the standard curve.

The concentrations of the serum and plasma samples have to be multiplied by 4.

The total amount of Citrulline excreted in urine during 24 h is calculated as following:

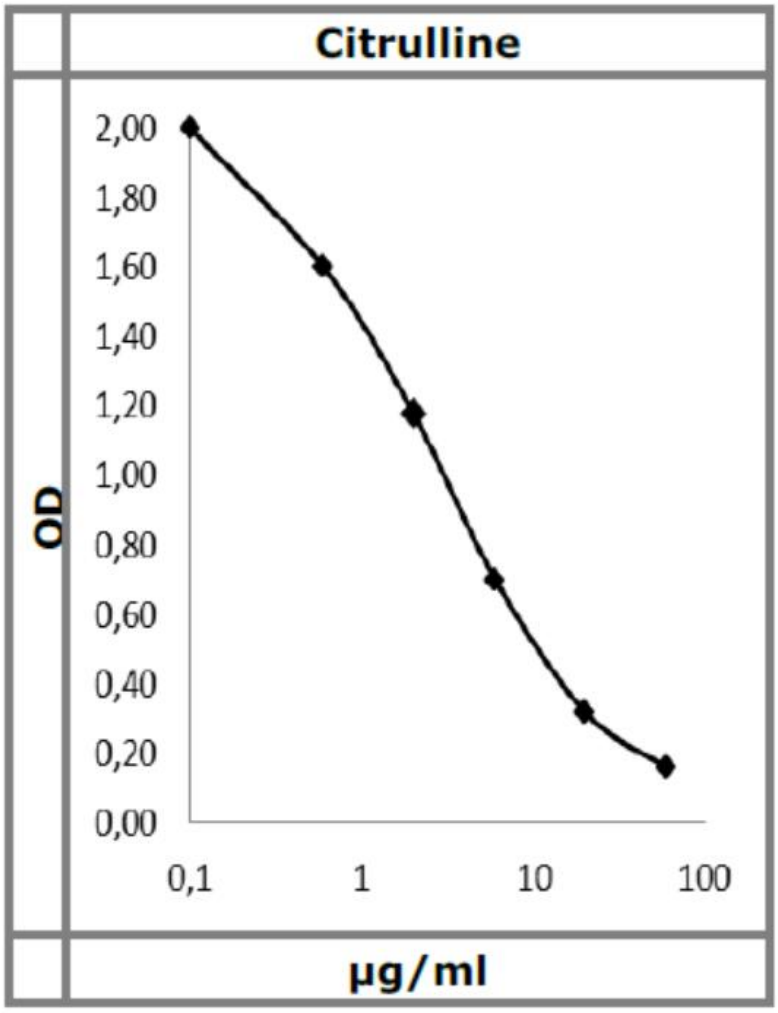
µg/24h = µg/ml x ml/24h

Conversion

Citrulline (µg/ml) x 5.71 = Citrulline (µmol/l)

Typical Standard Curve

Example, do not use for calculation!



Precision

		Sample	Mean concentration (µg/ml)	Inter Assay CV(%)
Precision: Inter Assay CV	Urine	1	1.3	13.4
		2	4.9	6.9
		3	10.1	5.4
	Plasma	1	2.2	6.9
		2	5.5	8.3
		3	10.9	5.9
	Serum	1	2.1	10.8
		2	5.4	17.0
		3	11.1	14.1

Detection Range

0.23 – 60 µg/ml

Sensitivity

0.23 µg/ml

Specificity

	Substance	Cross Reactivity (%)
		Citrulline
Analytical Specificity (Cross Reactivity)	DL-Citrulline	100
	L-Arginin	<0.001
	L-Glutamine	<0.001
	L-Ornithin	<0.001

Linearity

		Serial dilution up to	Mean Linearity (%)	Range Linearity (%)
Linearity	Urine	1:32	87	80 – 97
	Plasma	1:32	93	86 – 95
	Serum	1:32	93	86 – 101

Recovery

		Sample	Mean concentration (µg/ml)	Mean Recovery (%)
Recovery	Urine	1	1.54	85
		2	5.23	90
		3	10.6	98
	Plasma	1	2.28	93
		2	6.18	96
		3	10.6	93
	Serum	1	2.19	94
		2	5.79	92
		3	10.8	95

Interferences

Serum/Plasma

Samples containing precipitates or fibrin strands or which are haemolytic or lipemic might cause inaccurate results.

24- hour urine

Please note the sample preparation! If the percentage of the final concentration of acid is too high, this will lead to incorrect results for the urine samples.

Precautions

1. The kit is strictly intended for veterinary use only. Use by staff, who is specially informed and trained in methods which are carried out by use of immunoassays.
2. All blood components and biological materials should be handled as potentially hazardous in use and for disposal. Follow universal precautions when handling and disposing of infectious agents.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2-8°C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may result in colored solution. Do not pour reagents back into vials as reagent contamination may occur.

7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (18-25°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink, or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedure defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may vary slightly.
17. Avoid contact with Stop Solution. It may cause skin irritation and burns.
18. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline and regulation.
19. For information please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

Limitations

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to GLP (Good Laboratory Practice). Any improper handling of samples or modification of this test might influence the results.