Product Manual

Homocitrulline/Citrulline Assay Kit

Catalog Number

MET-5027 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Homocitrulline is an amino acid found in mammalian metabolism as a free-form metabolite of ornithine (another amino acid not found in proteins but is involved in the urea cycle). Through the process of carbamylation, homocitrulline amino acid residues can also be formed in proteins. Carbamylation results from the binding of isocyanic acid with amino groups (isocyanic acid spontaneously derived from high concentrations of urea) and primarily leads to the formation of either N-terminally carbamylated proteins and/or carbamylated lysine side chains (forming homocitrulline residues) (Figure 1A). It is known that elevated urea directly induces the formation of potentially atherogenic carbamylated LDL (cLDL). High blood concentrations of urea leading to the carbamylation process were detected in uremic patients and patients with end-stage renal disease. Homocitrulline can be detected in larger amounts in the urine of individuals with urea cycle disorders.

Citrulline is an amino acid very similar in structure to homocitrulline; however, the former is one methylene group shorter than the latter. In mammals, free citrulline is produced from free arginine during the enzymatic generation of nitric oxide (NO) by nitric oxide synthase (NOS) (Figure 1B). In addition, citrulline is synthesized from ornithine and carbamoyl phosphate in one of the main reactions of the urea cycle, a process that causes excretion of ammonia. Citrulline is not normally incorporated into proteins, but can be found in proteins due to post translational modification. The enzyme pepdidylarginine deiminase (PADI) can convert arginine to citrulline in the presence of calcium (Figure 1C). Since rheumatoid arthritis (RA) patients often produce autoantibodies to peptides containing citrulline, it has been suggested that PADI enzymes are involved in the disease. Recently it has been shown that haplotype PADI4 is associated with susceptibility to RA. Homocitrulline has been suggested as a confounding antigen for rheumatoid arthritis antibodies targeting citrullinated proteins/peptides. Antibodies binding to homocitrulline-containing sequences have been found in rheumatoid arthritis patients' sera. It has also been shown that homocitrulline-containing proteins are present in rheumatoid arthritis (RA) joints.

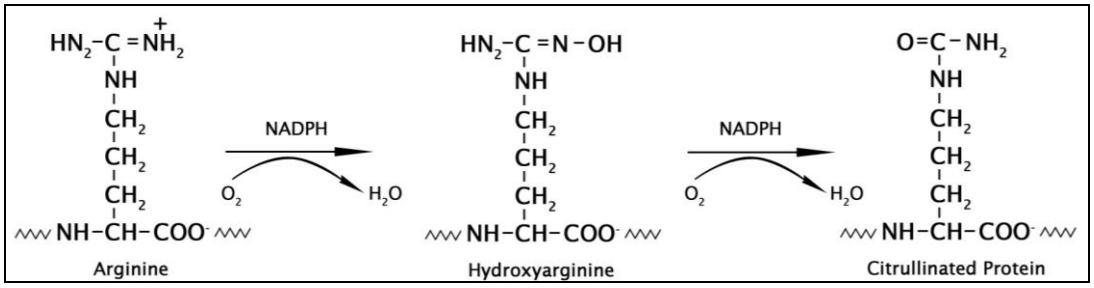
Cell Biolabs' Homocitrulline/Citrulline Assay Kit is a simple colorimetric assay that measures the amount of total homocitrulline and citrulline present in biological samples in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, homocitrulline or citrulline standards, and unknown samples. Sample homocitrulline/citrulline concentrations are determined by comparison with a known homocitrulline or citrulline standard. The kit has a detection sensitivity limit of 37.5 µM homocitrulline or citrulline.

Assay Principle

Cell Biolabs' Homocitrulline/Citrulline Assay Kit provides a convenient colorimetric method for the detection of total homocitrulline/citrulline from cells, tissue, plasma, serum, or urine samples. First, the samples are treated with sodium dodecyl sulfate (SDS) and Proteinase K to release free homocitrulline/citrulline residues. Assay Reagents are added to the well which reacts with homocitrulline and citrulline to produce a chromophore (Figure 2) and the absorbance is read at 540-560 nm. The content of homocitrulline and citrulline in the unknown samples is determined by comparison with a predetermined standard curve. The provided reagents are sufficient for the evaluation of 100 assays including standards and unknown samples.

A.

B.



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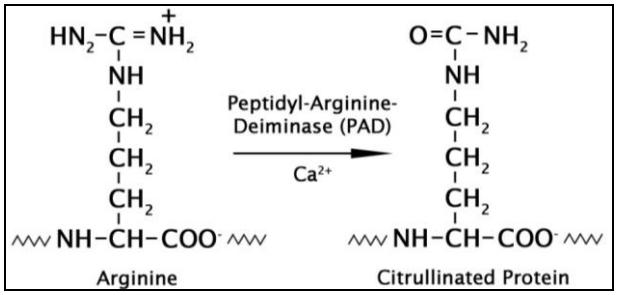

Figure 1. Mechanisms of **(A)** Homocitrullination (Carbamylation) of Proteins by Isocyanic Acid. **(B)** Citrulline Formation by NOS. **(C)** Citrullination of Proteins by PAD.

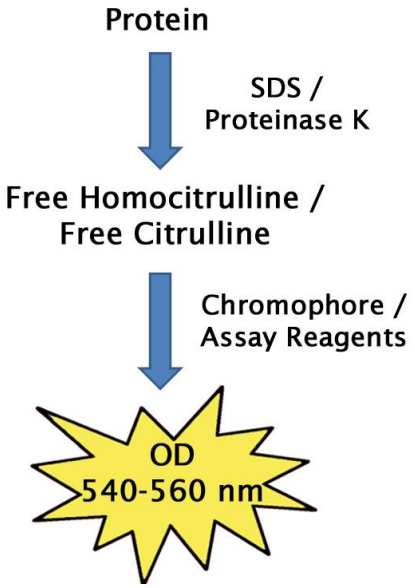

Figure 2. Assay principle.

Related Products

1. MET-5130: Lysine Assay Kit (Colorimetric)

2. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
3. MET-5136: D-Amino Acid Assay Kit (Colorimetric)
4. STA-877: OxiSelect™ Protein Carbamylation Sandwich Elisa Kit (Colorimetric)
5. STA-382: Urea Assay Kit (Colorimetric)

Kit Components

Box 1 (shipped at room temperature)

1. Homocitrulline Standard (Part No. 50271C): One 50 µL vial containing 240 mM Homocitrulline.
2. Citrulline Standard (Part No. 50272C): One 50 µL vial containing 240 mM Citrulline.
3. SDS Solution (Part No. 50273A): One 500 µL vial.
4. Assay Reagent A (Part No. 50275A): One 25 mL bottle.
5. Assay Reagent B (Part No. 50276A): One 5 mL bottle.

Box 2 (shipped on blue ice packs)

1. Proteinase K Solution (Part No. 50274C): One 500 µL vial.

Materials Not Supplied

1. Phosphate Buffered Saline (PBS)
2. 2 mL screwcap tubes with O-rings.
3. 96 well ELISA strips or 96 well microtiter plate

Storage

Upon receipt, store the Homocitrulline Standard, Citrulline Standard, and Proteinase K at -20°C. Store all the other reagents at room temperature.

Preparation of Standard Curve

Prepare a dilution series of Homocitrulline or Citrulline standards in the concentration range of 0 to 2400 µM by diluting the Homocitrulline or Citrulline Standard in PBS (Table 1).

Standard Tubes	240 mM Homocitrulline or Citrulline Standard (µL)	PBS (µL)	Homocitrulline or Citrulline (µM)
1	5	495	2400
2	250 of Tube #1	250	1200
3	250 of Tube #2	250	600
4	250 of Tube #3	250	300
5	250 of Tube #4	250	150
6	250 of Tube #5	250	75
7	250 of Tube #6	250	37.5
8	0	250	0

Table 1. Preparation of Homocitrulline or Citrulline Standards.

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Cell culture supernatants: Cell culture media formulated with homocitrulline or citrulline should be avoided. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The cell conditioned media may be assayed directly or diluted as necessary into PBS.
- Tissue lysates: Sonicate or homogenize tissue sample in PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. The supernatant may be assayed directly or diluted as necessary in PBS.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in PBS.
- Serum, plasma, or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed directly or diluted as necessary into PBS.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate.
2. Add 50 µL of each Homocitrulline standard, Citrulline standard or unknown sample into a 2 mL screwcap tube with an O-ring.
3. Add 5 µL of SDS solution and 5 µL of Proteinase K solution to each tube and mix thoroughly by pipetting up and down. Incubate for 2 hours at 37ºC.
4. Add 250 µL of Assay Reagent A and 50 µL of Assay Reagent B to each tube. Close all screwcap tubes tightly, mix well, and incubate for 30 minutes at 95°C.
5. Transfer the tubes to 4°C for 5 minutes. Centrifuge the tubes at 18,000 x g for 10 minutes at room temperature.
6. Transfer 200 µL of each supernatant to a new well of a clear 96 well plate or an ELISA strip well. Read absorbance of each well on a microplate reader using 540-560 nm as the primary wavelength.

Example of Results

The following figures demonstrate typical Homocitrulline/Citrulline Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

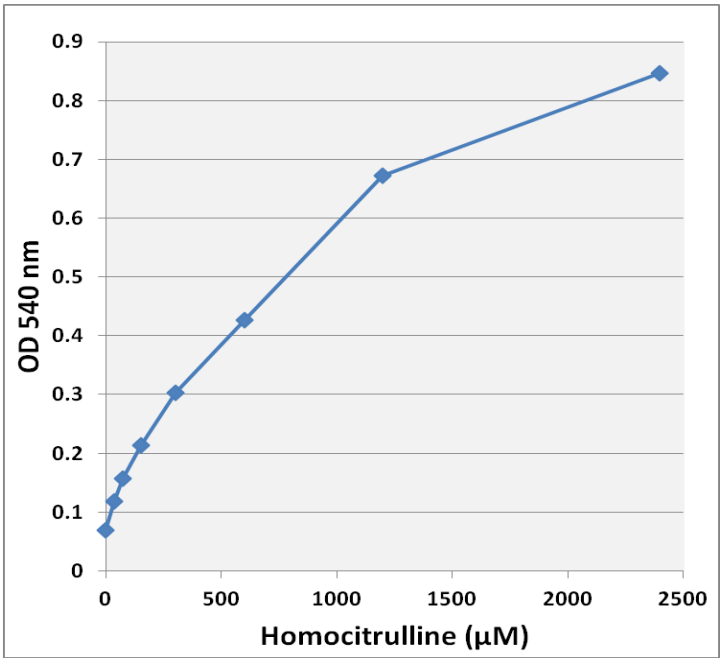

Figure 3. Homocitrulline standard curve.

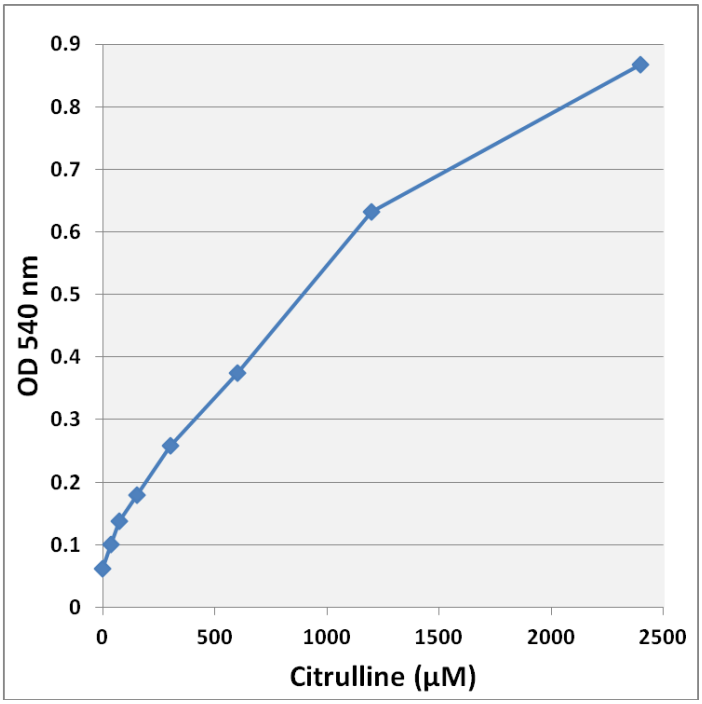

Figure 4. Citrulline standard curve.

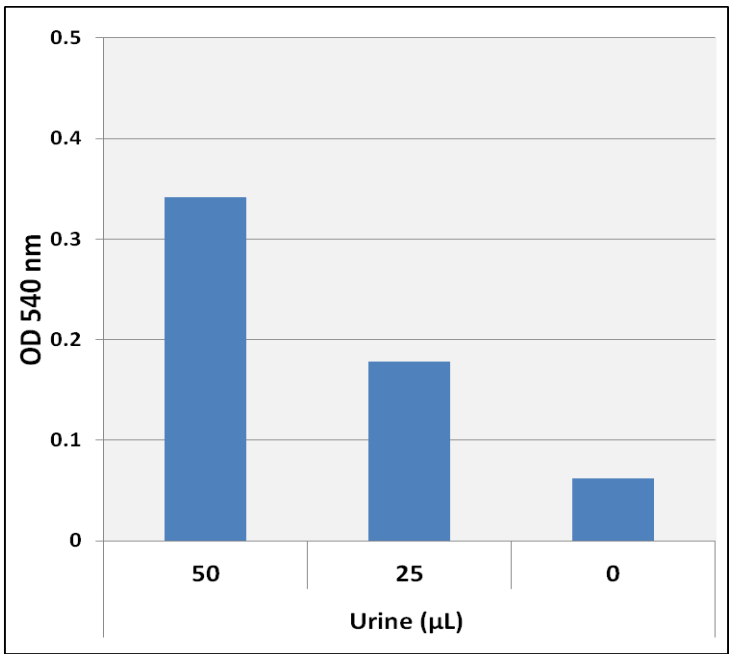

Figure 5. Detection of homocitrulline/citrulline in human urine.

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Recent Product Citations

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