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ab242292 Homocitrulline/Citrulline Assay Kit

For the detection of total homocitrulline/citrulline from cells, tissue, plasma, serum, or urine samples.

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

Table of Contents

1. Overview 3
2. Protocol Summary 4
3. General guidelines, precautions, and troubleshooting 5
4. Materials Supplied, and Storage and Stability 6
5. Materials Required, Not Supplied 6
6. Reagent Preparation 7
7. Assay Procedure 8
8. Typical Data 9
9. Notes 10

1. Overview

The Homocitrulline/Citrulline Assay Kit (ab242292) provides a convenient colorimetric method for the detection of total homocitrulline/citrulline from cells, tissue, plasma, serum, or urine samples.

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.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.

Add 50 µL standard or sample 2 mL screwcap tube with an O-ring

Add 5 µL of SDS solution and 5 µL of Proteinase K solution to each tube and mix. Incubate for 2 hours at 37°C.

Add 250 µL of Assay Reagent A and 50 µL of Reagent B to each tube. Mix and incubate for 30 minutes at 95°C.

Transfer the tubes to 4°C for 5 minutes. Centrifuge the tubes at 18,000 x g for 10 minutes at room temperature.

Transfer 200 µL of each supernatant to a new well. Read absorbance at 540/560nm.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
Homocitrulline standard	50 µL	-20°C
Citrulline standard	50 µL	-20°C
SDS Solution	500 µL	RT
Assay Reagent A	25 mL	RT
Assay Reagent B	5 mL	RT
Proteinase K Solution	500 µL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Water bath, heat block, or incubator capable of heating to 37°C and 95 °C
- 2 mL screwcap tubes with O-rings.
- 96 well ELISA strips or 96 well microtiter plate
- 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Microplate reader capable of reading at 540-560 nm

6. Reagent Preparation

- All components are ready to use as supplied.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- 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, as shown in the table below.

Standard #	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

8. Sample Preparation

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

8.1 Cell culture Supernatant:

– 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.

8.2 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.

8.3 Cell Lysates:

– Resuspend cells at 1-2 x 106 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.

8.4 Serum Plasma and 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.

9. Assay Procedure

9.1 Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate.

9.2 Add 50 µL of each Homocitrulline standard, Citrulline standard or unknown sample into a 2 mL screwcap tube with an O-ring.

9.3 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 37°C.

9.4 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.

9.5 Transfer the tubes to 4°C for 5 minutes. Centrifuge the tubes at 18,000 x g for 10 minutes at room temperature.

9.6 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.

10. Typical Data

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

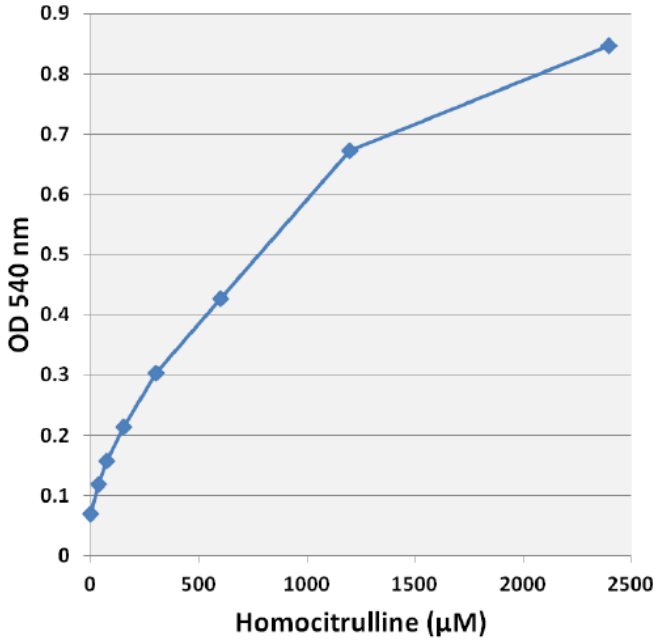

Figure 1. Homocitrulline standard curve.

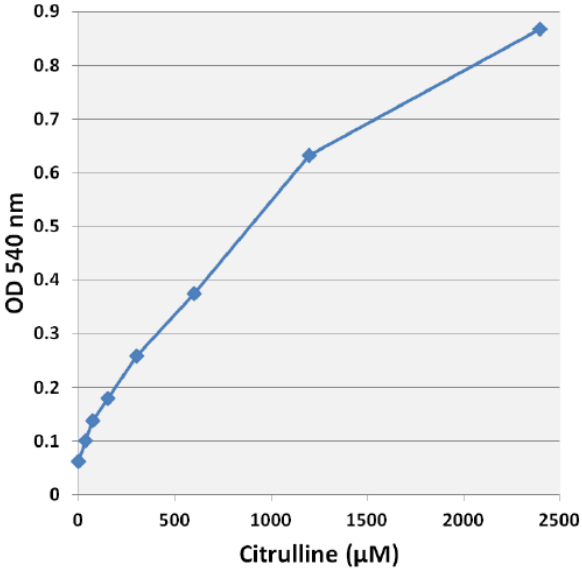

Figure 2: Citrulline standard curve.

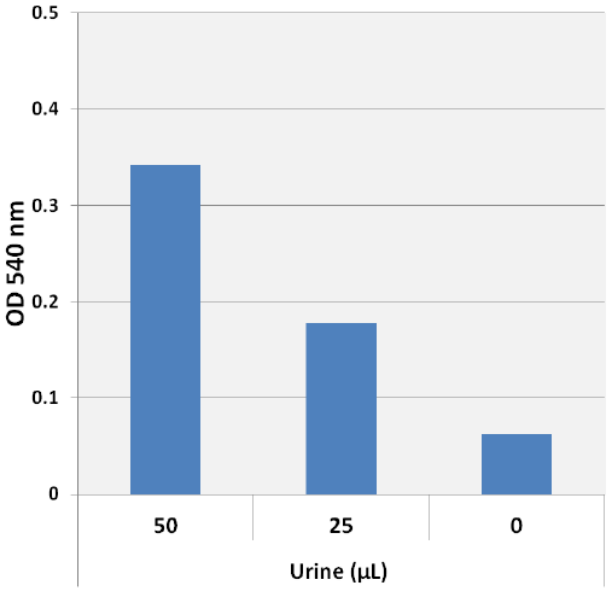

Figure 3: Detection of homocitrulline/citrulline in human urine.

11. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)