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ab273309
Citrulline Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273309
(use https://www.abcam.cn/ab273309 for china, or
https://www.abcam.co.jp/ab273309 for Japan)

For the determination of Citrulline in biological samples and fruit juices.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

Citrulline Assay Kit (Fluorometric) (ab273309) provides a rapid, specific, and easy method for the measurement of total Citrulline concentrations in a wide variety of samples.

In this enzymatic assay, Citrulline is converted into a series of intermediates, which further reacts with a probe producing a stable fluorescent signal (Ex/Em = 535/587 nm). The kit is simple, easy to perform, sensitive and is high throughput adaptable.

It can detect as low as 2 µM Citrulline in biological samples.

2. Protocol Summary

Prepare sample, e.g. fruit juice or biological samples.

Prepare all reagents as directed.

Prepare standards.

Add all Samples, Controls and Standards to appropriate wells and adjust volume to 50 µL.

Add Reaction Mix and Background Mix to the appropriate wells. Incubate the plate for 30 mins at 37°C protected from light.

Measure fluorescence in end-point mode.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
Assay Buffer LXV/Citrulline Assay Buffer	25 mL	-20°C
Buffer Supplement I/Citrulline Buffer Supplement (Lyophilized)	1 vial	-20°C
Citrulline Converter Mix/Citrulline Converter Mix (Lyophilized)	1 vial	-20°C
Development Enzyme Mix III/Citrulline Developer Mix	200 µL	-20°C
Citrulline Cofactor Mix	200 µL	-20°C
Development Enzyme Mix I/Citrulline Enzyme Mix (Lyophilized)	1 vial	-20°C
OxiRed Probe/Citrulline Probe	200 µL	-20°C
Citrulline Standard/Citrulline Standard (Lyophilized)	1 vial	-20°C

7. Materials Required, Not Supplied

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

- 96-well black plate with flat bottom
- Multi-well spectrophotometer
- dH_2O

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer LXV/Citrulline Assay Buffer:**
Store at -20 °C. Bring to room temperature before use.

9.2 **Development Enzyme Mix III/Citrulline Developer Mix:**
Thaw on ice. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze and thaw cycles. Use within two months.

9.3 **Citrulline Cofactor Mix:**
Thaw on ice. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze and thaw cycles. Use within two months.

9.4 **Buffer Supplement I/Citrulline Buffer Supplement (Lyophilized):**
Reconstitute the vial with 220 µl Assay Buffer LXV/Citrulline Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze and thaw cycles. Use within two months.

9.5 **Citrulline Converter Mix/Citrulline Converter Mix (Lyophilized):**
Reconstitute the vial with 220 µl Assay Buffer LXV/Citrulline Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze and thaw cycles. Use within two months.

9.6 **Development Enzyme Mix I/Citrulline Enzyme Mix (Lyophilized):**
Reconstitute the vial with 220 µl Assay Buffer LXV/Citrulline Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze and thaw cycles. Use within two months

9.7 **Citrulline Standard/Citrulline Standard (Lyophilized):**
Reconstitute with 100 µl of dH_2O to make 100 mM Citrulline Standard stock solution. Store at -20°C.

10. Sample Preparation

- For all samples, prepare 2 additional wells with 50 µl Assay Buffer LXV/Citrulline Assay Buffer labeled as Blank (B) and Reagent Control (RC).
- Citrulline varies over a wide range based on the Sample type. For Unknown Samples, we recommend performing a pilot experiment with a few dilutions to ensure reading are within the Standard Curve range.
- For Watermelon Juice, average Citrulline concentration ranges from 10-20 mM. For normal human serum, average Citrulline concentration is 6-70 µM. Citrulline can range to 30-3000 µM for patients with Citrullinemia or Argininosuccinic Aciduria.

For fruit juices and beverages:

10.1 Centrifuge Samples at 13,000 *x g* to remove any insoluble precipitate.

10.2 Collect the supernatant and put 200-500 µl into a 10 kDa MWCO Spin Column.

10.3 Centrifuge the Sample at 13,000 *x g* and 4°C for 10 mins and collect the filtrate.

10.4 Add 2-50 µl of the filtered Sample and label as Sample and Sample Background Control (SBC) into two parallel wells of a 96-well black plate.

10.5 Make up the volume to 50 µl with Assay Buffer LXV/Citrulline Assay Buffer.

For biological fluids:

10.6 Centrifuge at 13,000 *x g* and 4°C for 10 mins to remove any insoluble precipitate in the biological fluids.

10.7 Add 200-500 µl of Sample into a 10 kDa MWCO Spin Column.

10.8 Centrifuge the Sample at 10,000 *x g* and 4°C for 20 mins and collect the filtrate. Due to matrix effect in biological samples, an Internal Standard (Spike) is needed for each Test Sample.

10.9 For each Test Sample, add 2-50 µl of Samples into 3 wells of a 96-well black plate.

10.10 Label each well as Sample, Sample Background Control (SBC) and Spike (SP).

10.11 Add 4 µl of 0.1 mM Citrulline Standard (i.e. 400 pmol) into the SP wells.

10.12 Bring the volume of all wells to 50 µl/well with Assay Buffer LXV/Citrulline Assay buffer.

11. Standard Curve

11.1 Dilute Citrulline Standard to 1 mM by adding 10 µl of the 100 mM Citrulline Standard into 990 µl of dH_2O.

11.2 Dilute the 1 mM Citrulline Standard solution to 0.1 mM Standard solution by adding 10 µl of the 1 mM Citrulline Standard into 90 µl of dH_2O.

11.3 Add 0, 2, 4, 6, 8, 10 µl of the 0.1 mM Citrulline Standard into a series of wells to generate 0, 200, 400, 600, 800, 1000 pmol of Citrulline/well of a 96 well plate.

11.4 Adjust the volume to 50 µl/well with the Assay Buffer LXV/Citrulline Assay buffer.

Standard #	0.1 mM Citrulline -Standard (µL)	Assay Buffer (µL)	Citrulline (pmol/well)
1	10	40	1000
2	8	42	800
3	6	44	600
4	4	46	400
5	2	48	200
6	0	50	0

Δ Note: Do not store the diluted Standards.

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

Reaction mix:

12.1 Prepare a 5-fold dilution of the OxiRed Probe/Citrulline Probe by mixing 5 µl of OxiRed Probe/Citrulline Probe with 20 µl Assay Buffer LXV/Citrulline Assay Buffer.

12.2 For each well, prepare 50 µl of the Substrate Mix:

Component	Reaction Mix (µl)	Background Mix (µl)
Assay Buffer LXV/Citrulline Assay Buffer	38	40
Buffer Supplement I/Citrulline Buffer Supplement	2	2
Citrulline Converter Mix	2	---
Development Enzyme Mix III/Citrulline Developer Mix	2	2
Citrulline Cofactor Mix	2	2
Development Enzyme Mix I/Citrulline Enzyme Mix	2	2
Diluted OxiRed Probe/Citrulline Probe	2	2

12.3 Mix and add 50 µl of the Reaction Mix to each well(s) containing B, S and SP.

12.4 Add 50 µl of the Background Mix into SBC and RC wells.

12.5 Mix well and incubate the plate for 30 mins at 37°C protected from light.

12.6 Measure = fluorescence (Ex/Em=535/587 nm) in a microplate reader in endpoint mode.

13. Calculations

13.1 Subtract 0 Standard reading from all readings.
13.2 Plot the Citrulline -Standard Curve and obtain the slope of the curve (ΔRFU/pmol).
13.3 If Sample Background Control reading is significant then subtract the Background Control reading from Sample readings.
13.4 To calculate the specific Citrulline activity of Sample, subtract ΔRFU of Negative Control (ΔRFU_{NC}) from Sample (ΔRFU_S).

For Spiked samples: Amount of Citrulline in Sample wells (C) =

$$\frac{FS - FB}{FSP - FS} \times Citrulline\ Spike\ (pmol)$$

For biological fluids: Citrulline concentration =

$$\frac{C}{V} \times D = pmol$$

V = Volume of Sample added to the well (in µl)
D = Sample dilution factor (D= 1 for undiluted Samples)

Δ Note: If calculated citrulline amount in the spiked well(s) is higher than 600 pmol dilute further the sample.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

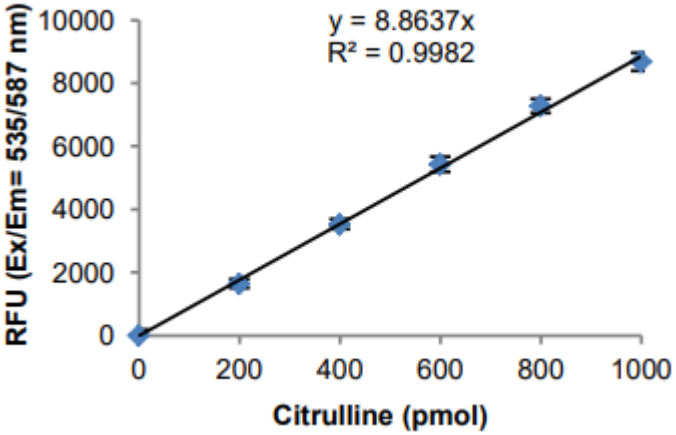

Figure 1. Citrulline Standard Curve.

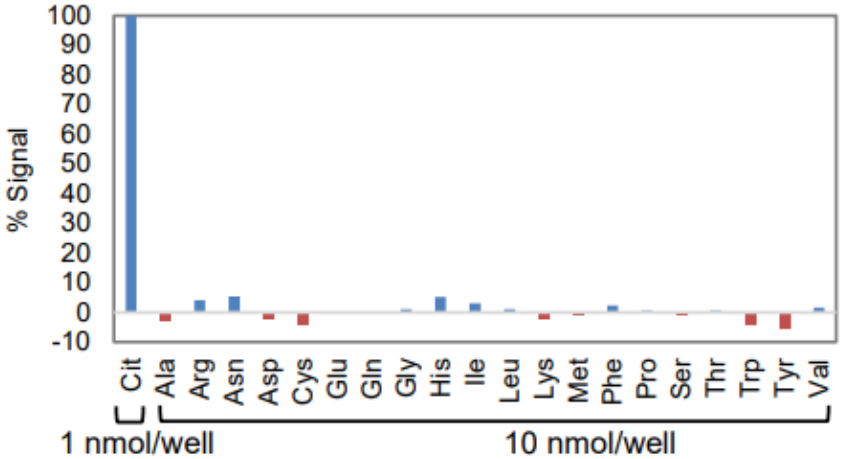

Figure 2. Specificity of the detection of Citrulline over other amino acids. Other amino acids were tested at a 10-fold molar excess (each AA: 10 nmol) vs Citrulline (1 nmol).

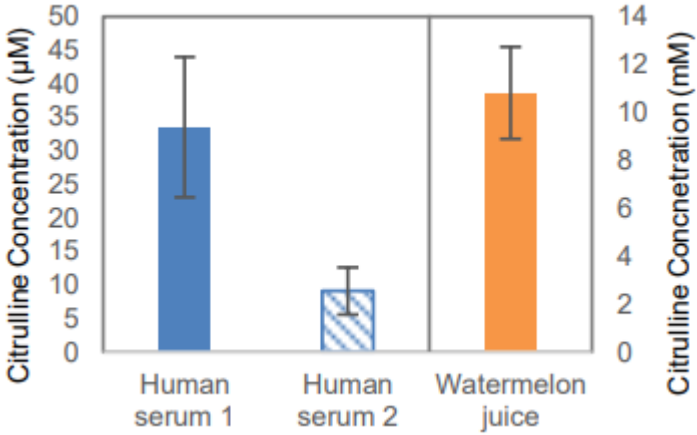

Figure 3. Estimations of Citrulline in 2 human serum samples (10 and 40 µl in each well respectively) and watermelon juice (4 µl of 100X dilution). Citrulline concentrations were 33.50 µM and 9.11 µM in human serum respectively and 10.81 mM in watermelon juice.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. 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)