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ab273309 Citrulline Assay Kit (Fluorometric)

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<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.

Table of Contents

1. Overview	1
2. Protocol Summary	2
3. Precautions	3
4. Storage and Stability	3
5. Limitations	4
6. Materials Supplied	4
7. Materials Required, Not Supplied	5
8. Technical Hints	5
9. Reagent Preparation	6
10. Sample Preparation	7
11. Standard Curve	8
12. Assay Procedure	9
13. Calculations	10
14. Typical Data	11
15. FAQ / Troubleshooting	12
16. Notes	13

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 μ M 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 μ L.



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 45	25 mL	-20°C
Buffer Supplement I	1 vial	-20°C
Citrulline Converter Mix	1 vial	-20°C
Developer Mix C	200 µL	-20°C
Citrulline Cofactor Mix	200 µL	-20°C
Developer Mix A	1 vial	-20°C
OxiRed™ Probe	0.2 mL	-20°C
Citrulline Standard	1 vial	-20°C

PLEASE NOTE: Developer Mix A was previously labelled as Development Enzyme Mix I and Citrulline Enzyme Mix (Lyophilized), and Developer Mix C as Development Enzyme Mix III and Citrulline Developer Mix. Also, Assay Buffer 45 was previously labelled as Assay Buffer LXV and Citrulline Assay Buffer, and OxiRed™ Probe as OxiRed Probe and Citrulline Probe. The composition has not changed.

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
- dH₂O

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 45:**

Store at -20 °C. Bring to room temperature before use.

9.2 **Developer Mix C:**

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:**

Reconstitute the vial with 220 µl Assay Buffer 45. 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:**

Reconstitute the vial with 220 µl Assay Buffer 45. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze and thaw cycles. Use within two months.

9.6 **Developer Mix A:**

Reconstitute the vial with 220 µl Assay Buffer 45. 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:**

Reconstitute with 100 µl of dH₂O to make 100 mM Citrulline Standard stock solution. Store at -20°C.

10. Sample Preparation

- For all samples, prepare 2 additional wells with 50 μ l Assay Buffer 45 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 μ M. Citrulline can range to 30-3000 μ M 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 μ l 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 μ l of the filtered Sample and label as Sample and Sample Background Control (SBC) into two parallel wells of a 96-well black plate.
Make up the volume to 50 μ l with Assay Buffer 45.

For biological fluids:

- 10.5 Centrifuge at 13,000 x g and 4°C for 10 mins to remove any insoluble precipitate in the biological fluids.
- 10.6 Add 200-500 μ l of Sample into a 10 kDa MWCO Spin Column.
- 10.7 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.
- 10.8 For each Test Sample, add 2-50 μ l of Samples into 3 wells of a 96-well black plate.
- 10.9 Label each well as Sample, Sample Background Control (SBC) and Spike (SP).
- 10.10 Add 4 μ l of 0.1 mM Citrulline Standard (i.e. 400 pmol) into the SP wells.
- 10.11 Bring the volume of all wells to 50 μ l/well with Assay Buffer 45.

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 45.

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 by mixing 5 µl of OxiRed™ Probe with 20 µl Assay Buffer 45.
- 12.2 For each well, prepare 50 µl of the Substrate Mix:

Component	Reaction Mix (µl)	Background Mix (µl)
Assay Buffer 45	38	40
Buffer Supplement I	2	2
Citrulline Converter Mix	2	---
Developer Mix C	2	2
Citrulline Cofactor Mix	2	2
Developer Mix A	2	2
Diluted OxiRed™ 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 ($\Delta\text{RFU}/\text{pmol}$).
- 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 ($\Delta\text{RFU}_{\text{NC}}$) from Sample ($\Delta\text{RFU}_{\text{S}}$).

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

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

For biological fluids: Citrulline concentration =

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

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.

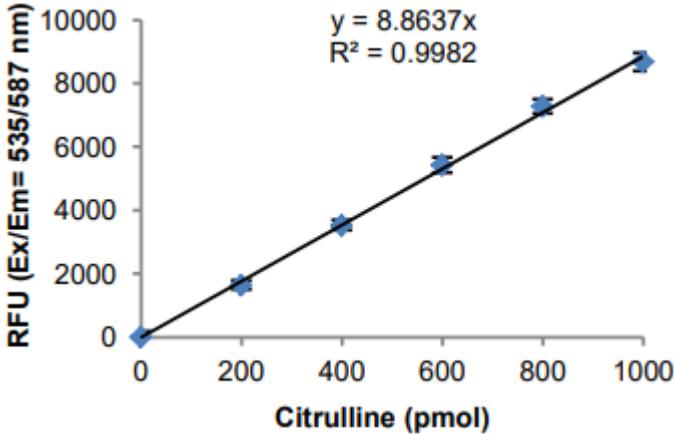


Figure 1. Citrulline Standard Curve.

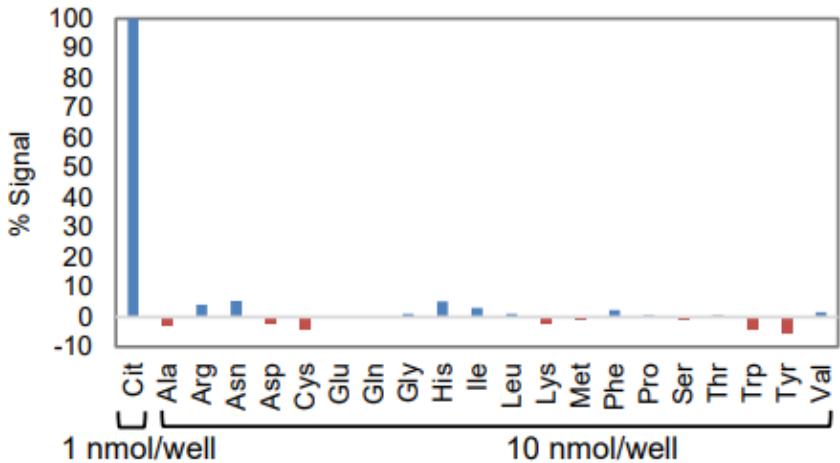


Figure 2. Specificity of the detection of Citrulline over other amino acids. Other amino acids were tested at a 10-fold molar excess (each AA: 10 nmol) vs Citrulline (1 nmol).

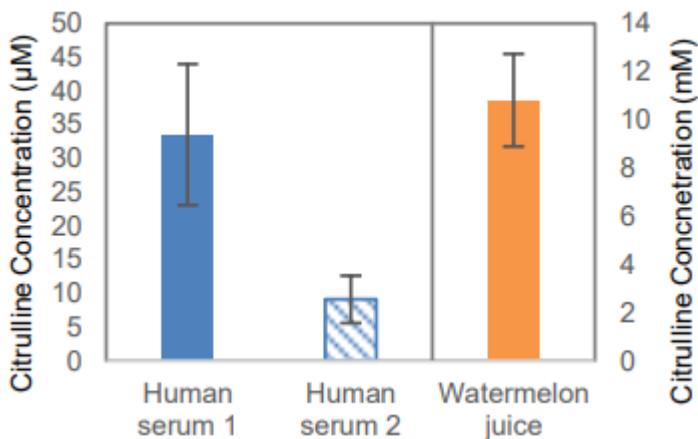


Figure 3. Estimations of Citrulline in 2 human serum samples (10 and 40 µl in each well respectively) and watermelon juice (4 µl of 100X dilution). Citrulline concentrations were 33.50 µM and 9.11 µM in human serum respectively and 10.81 mM in watermelon juice.

15. FAQ / Troubleshooting

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

16. Notes

Technical Support

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