



Citrulline Fluorometric Assay Kit

(Catalog #K2002-100; 100 assays; Store at -20 °C)

I. Introduction:

L-Citrulline is a non-proteogenic, semi-essential amino acid. Citrulline is formed either by ornithine carbamoyltransferase or as a byproduct of the Nitric oxide synthase (NOS) activity. Citrulline generated in the NOS reaction can be recycled to arginine by the two enzymes argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) acting in the Urea Cycle. Citrulline concentrations have been shown to be elevated in patients with mutated ASS or ASL genes. Watermelon is one of the richest sources of Citrulline. Additionally, Citrulline has been advertised as sports nutrition supplement due to its involvement in Nitric Oxide (NO) synthesis, which helps with vasodilation. BioVision's Citrulline Assay Kit 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 highthroughput adaptable. It can detect as low as 2 μ M Citrulline in biological samples.



Fluorometric Detection (Ex/Em = 535/587 nm)

II. Applications:

- Measurement of Citrulline in beverages and biological samples.
- Analysis of Urea cycle and NO cycle.

III. Sample Type:

- Biological fluids such as serum
- Fruit juices

IV. Kit Contents:

Components	K2002-100	Cap Code	Part Number
Citrulline Assay Buffer	25 ml	WM	K2002-100-1
Citrulline Buffer Supplement	1 vial	Orange	K2002-100-2
Citrulline Converter Mix	1 vial	Purple	K2002-100-3
Citrulline Developer Mix	200 µl	Blue	K2002-100-4
Citrulline Cofactor Mix	200 µl	White	K2002-100-5
Citrulline Enzyme Mix	1 vial	Green	K2002-100-6
Citrulline Probe	200 µl	Red	K2002-100-7
Citrulline Standard	1 vial	Yellow	K2002-100-8

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- Multi-well spectrophotometer
- dH₂O

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials prior to opening. Read the protocol before performing the assay.

- Citrulline Assay Buffer: Warm to Room temperature (RT) before use. Store at -20 °C.
- Citrulline Developer Mix, 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.
- Citrulline Buffer Supplement, Citrulline Converter Mix, Citrulline Enzyme Mix: Reconstitute each vial with 220 µl Citrulline Assay Buffer. Aliquot and store at -20 °C. Keep on ice while in use. Avoid freeze and thaw cycles. Use within two months.
- Citrulline Probe (in DMSO): Ready to use as supplied. Warm to RT before use. Store at 4°C or -20 °C. Keep away from light.
- Citrulline Standard: Reconstitute with 100 µl of dH₂O to make 100 mM Citrulline Standard stock solution. Store at -20 °C.

VII. Citrulline Assay Protocol:

1. Sample Preparation: For fruit juices and beverages: Centrifuge Samples at 13,000 x g to remove any insoluble precipitate. Collect the supernatant and put 200-500 μl into a 10 kDa MWCO Spin Column (Cat# 1997). Centrifuge the Sample at 13,000 x g and 4°C for 10 min, and collect the filtrate. Add 2-50 μl of the filtered Sample and label as "Sample (S)" and "Sample Background Control (SBC)" into two parallel wells of a 96-well black plate. Make up the volume to 50 μl with Citrulline Assay Buffer.

For biological fluids: Centrifuge at 13,000 x g and 4 °C for 10 min to remove any insoluble precipitate in the biological fluids. Add 200-500 µl of Sample into a 10 kDa MWCO Spin Column (Cat# 1997). Centrifuge the Sample at 10,000 x g and 4 °C for 20 min, and collect the filtrate. Due to matrix effect in biological samples, an Internal Standard (Spike) is needed for each Test Sample. For each Test Sample, add 2-50 µl of Samples into 3 wells of a 96-well black plate. Label each well as "Sample (S)", "Sample Background Control (SBC)" and "Spike (SP)". Add 4 µl of 0.1 mM Citrulline Standard (i.e 400 pmol) into the "SP" wells. Bring the volume of all wells to 50 µl/well with Citrulline Assay buffer.

For all samples, prepare 2 additional wells with 50 µl Citrulline Assay Buffer labeled as "Blank (B)" and "Reagent Control (RC)".

Notes:

a. 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.

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b. 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.

- 2. Standard Curve Preparation: Dilute Citrulline Standard to 1 mM by adding 10 µl of the 100 mM Citrulline Standard into 990 µl of dH₂O. 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 dH₂O. 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. Adjust the volume to 50 µl/well with the Citrulline Assay buffer. *Do not store the diluted Standards.*
- **3. Reaction Mix:** Mix enough reagents for the number of assays to be performed. Prepare a 5-fold dilution of the Citrulline Probe by mixing 5 µl of Citrulline Probe with 20 µl Citrulline Assay Buffer. For each well, prepare 50 µl Mix as mentioned below:

	Reaction Mix	Background Mix
Citrulline Assay Buffer	38 µl	40 µl
Citrulline Buffer Supplement	2 µl	2 µl
Citrulline Converter Mix	2 µl	-
Citrulline Developer Mix	2 µl	2 µl
Citrulline Cofactor Mix	2 µl	2 µl
Citrulline Enzyme Mix	2 µl	2 µl
Diluted Citrulline Probe	2 µl	2 µl

Mix and add 50 µl of the Reaction Mix to each well containing Blank, Standard, Sample and Spike. Add 50 µl of the Background Mix into Sample Background Control and Reagent Control wells. Mix well and incubate the plate for 30 min at 37 °C, protected from light.

- 4. Measurement: Measure fluorescence (Ex/Em=535/587 nm) in a microplate reader in endpoint mode.
- 5. Calculation: Subtract 0 Standard reading from all Standard readings. Plot the Citrulline Standard Curve. Subtract RC readings from Blank ($F_B = RFU_B RFU_B RFU_{RC}$). Subtract SBC readings from S readings ($F_S = RFU_S RFU_{SBC}$) and SP readings ($F_{SP} = RFU_{SP} RFU_{SBC}$) respectively. For Unspiked Samples, calculate the Citrulline amount (C) from the Citrulline Standard Curve.

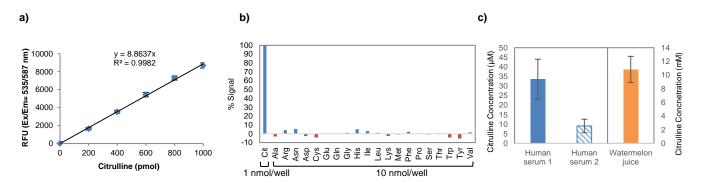
For Spiked Samples: Amount of Citrulline in Sample wells (C) = $\frac{F_S - F_B}{F_{SP} - F_S} \times$ Citrulline Spike (in pmol)

For biological fluids: Sample Citrulline Concentration = $\frac{c}{u} \times D$ = pmol/ µl = µM

Where: V is the volume of Sample added to the well (in µl)

D is the Sample dilution factor (D= 1 for undiluted Samples)

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



Figures: a). Citrulline Standard Curve. b). 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). c). Estimations of Citrulline in 2 human serum samples (10 and 40 μ l in each well respectively) and watermelon juice (4 μ l of 100X dilution). Citrulline concentrations were 33.50 μ M and 9.11 μ M in human serum respectively and 10.81 mM in Watermelon Juice. Assays were performed following the kit protocol.

VIII. Related Products:

DL-serine Kit (K545) Alanine Kit (K652) Cysteine Kit (K558) L-amino acid kit (K639) Threonine Kit (K463) Glycine Kit (K589) Phenylalanine Kit (K572) Tyrosine Kit (K573) Methionine Kit (K442) Tryptophan Kit (K557) Glutamate Kit (K629) Glutamine Kit (K556) Aspartate Kit (K552) Homocysteine Kit (K531) Total D-amino acid Kit (K445) Arginine (Colorimetric) Kit (K749) Ornithine Kit (K939) Taurine Kit (K988)

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