

Colorimetric L-Citrulline – Determination

K 6600

Sample preparation

- In 1.5 mL micro-reactors, pipette 100 μ L of the sample dilution (SAMDIL) and 500 μ L of sample.
- Mix vigorously.
- Incubate at 37°C for 1 hour.
- Pipette 150 μ L of cold (2–8°C) precipitating reagent (PREC) into the micro-reactors.
- Mix vigorously.
- Incubate at 2–8°C for 30 minutes.
- Place micro-reactors in centrifuge for 10 minutes at 20000 x g at 4°C
- Use supernatant for assay

Preparation of the standard-curve

Prepare a calibration curve from the L-Citrulline-stock-solution (STD) as follows:

- Dilute L-Citrulline stock (40 mmol/L) 1:100 with the standard-dilution buffer (STDBUF) for standard 1 (400 μ mol/L)
- For standard 2 (200 μ mol/L): dilute standard 1 (400 μ mol/L) 1:2 with STDBUF
- For standard 3 (100 μ mol/L): dilute standard 2 (200 μ mol/L) 1:2 with STDBUF
- For standard 4 (50 μ mol/L): dilute standard 3 (100 μ mol/L) 1:2 with STDBUF
- For standard 5 (25 μ mol/L): dilute standard 4 (50 μ mol/L) 1:2 with STDBUF
- For standard 6 (12,5 μ mol/L): dilute standard 5 (25 μ mol/L) 1:2 with STDBUF
- For standard 7 (6,25 μ mol/L): dilute standard 6 (12,5 μ mol/L) 1:2 with STDBUF
- For standard 8 use standard-dilution buffer only.

Preparation of the color development solution:

- Mix 1 part solution A (SOL A) with 3 parts solution B (SOL B). (New color development solution must be prepared each time the test is performed and mixed just prior to use, since the solution only will remain active for about 30 minutes).
- Solutions A and B should be stored in a refrigerator (2–8°C) but should be near room temperature when used for this.



Test procedure:

- Pipette 2 x 60 µL of standard curve into micro-titer well (2 wells per standard curve point, 60 µL each).
- Pipette 4 x 60 µL of prepared sample into micro-titer well (4 wells per sample, 60 µL each).
- Pipette 200 µL of color development solution to all the standard curve wells and to 2 of the sample wells.
- Pipette 200 µL of solution B to the remaining two sample wells.
- Transfer the micro-titer strips to a metal holder preheated to 90°C.
- Seal micro-titer wells with adhesive tape.
- Incubate at 90°C for 15 minutes.
- Remove plate from oven and transfer micro-titer strips back to the original holder.
- Let cool at room temperature for 10 minutes (samples will remain stable 30 minutes after removal from oven).
- Measure optical density with ELISA reader using a 540 nm filter.

Calculation and Results

- The final concentration of L-Citrulline in µmol/L is the difference between the concentration of the sample with color development solution and the concentration of the sample with solution B times 1,5 or:

$$([\text{sample with color development solution}] - [\text{sample without solution B}]) \times 1,5 = [\text{L-Citrulline } [\mu\text{mol/L}]]$$

Literature:

Knipp M, Vasak M (2000) A Colorimetric 96-well Microtiter Plate Assay for the Determination of Enzymatically Formed Citrulline. Analytical Biochemistry 286, 257-264

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