EnzyChrom™ L-Arginine Assay Kit (EAGN-100)

Quantitative Colorimetric L-Arginine Determination

DESCRIPTION

L-ARGININE is an amino acid essential for protein metabolism and primarily found in the active sites of various proteins. During nitrogen metabolism, arginase breaks down arginine into urea and ornithine. L-Arginine is also present in many protein-rich foods and beverages. In particular, its detection in beverages, such as juices and wine, is crucial for quality control.

BioAssay Systems' L-Arginine assay measures the urea generated from arginase hydrolysis of arginine with our proprietary reagents. The increase in absorbance at 450 nm is proportional to the arginine concentration.

KEY FEATURES

Simple. Using our internal standard method and robust reagents, no sophisticated sample pretreatment is required.

Sensitive and accurate. Use as little as 10 µL samples. Linear detection range in 96-well plate: 0.066 to 3 mM for colorimetric assays.

High-throughput. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of arginine in food, beverage, biological samples (e.g. serum, cell lysate, etc).

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 5 mL **Enzyme Mix:** 100 µL
Standard: 400 µL 20mM **Mn Solution** 300 µL
Reagent A: 12 mL **Reagent B:** 12 mL

Storage conditions. The kit is shipped on ice. Upon receiving, store kit at -20°C (Mn Solution, Reagent A and B can be stored at 4°C). Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation. Prior to assays, all samples should be clear and free of turbidity or precipitates, e.g. by filtration or centrifugation (5 min at 14,000 rpm) and neutralized to pH 6.5 - 8.0.

Most samples, e.g. juice, food, beverage, serum, cell and tissue lysates, exhibit matrix inhibitory effects and require an internal standard for assay.

For samples with unknown arginine concentrations, it is prudent to test several dilutions in H2O to determine an optimal sample dilution factor *n*.

Reagent Preparation. Keep thawed Enzyme Mix on ice and equilibrate all other reagents to 25°C. Briefly centrifuge tubes before use.

Internal Standard Method

(A). Sample preparation. Each sample requires three separate assay wells: (1) Sample Blank, (2) Sample, and (3) Internal Standard ("IS").
For (1) and (2), add 10 µL of sample to two separate wells of a clear 96-well plate. For (3) the *IS* well, mix 1 µL of 20 mM Standard with 40 µL of sample, and transfer 10 µL to a separate well (0.5 mM arginine).

(B). Arginase Reaction: For wells (2) and (3), prepare enough Working Reagent (WR) by mixing, for each well: 42 µL Assay Buffer, 1 µL Enzyme Mix, and 0.5 µL Mn Solution. For the sample blank wells (1), prepare Blank Working Reagent (BWR) by mixing all the components of the WR except Enzyme Mix.

Add 40 µL WR per well to the sample (2) and *IS* (3) wells. Add 40 µL WRo to the Sample Blank wells (3). Tap plate to mix briefly and thoroughly.

Incubate reaction plate at 37°C for 30 min.

(C). Urea Determination: Prepare *Urea Reagent* for all assay wells by combining equal volumes of Reagent A and Reagent B. Add 150 µL *Urea Reagent* to each assay well. (note: Urea Reagent stops arginase reaction). Tap plate briefly to mix. Read OD_{450nm} at room temperature immediately (OD_0) and at 60 min (OD_{60}), or record kinetics for 60 min.

Standard Curve Method: Use this method only if the sample contains solely arginine and is not in a complex biological matrix.
Prepare 3.0 mM Arginine Premix by mixing 30 µL 20 mM Standard and 170 µL distilled water. Dilute standard as follows.

No	Premix + H_2O	Vol (µL)	Arginine (mM)
1	100 µL + 0 µL	100	3.0
2	60 µL + 40 µL	100	1.8
3	30 µL + 70 µL	100	0.9
4	0 µL + 100 µL	100	0.0

Transfer 10 µL standards and 10 µL samples into separate wells of a clear 96-well plate.
Follow the above protocol with (B) Arginase Reaction with 40 µL WR, and (C) Urea Determination.

CALCULATION

For the Internal Standard Method, compute L-Arginine concentration as follows:

$$[\text{L-Arginine}] = \frac{\Delta OD_{SAMPLE} - \Delta OD_{BLANK}}{\Delta OD_{IS} - \Delta OD_{SAMPLE}} \times n \times 0.5 \quad (\text{mM})$$

where ΔOD_{BLANK}, ΔOD_{SAMPLE} and ΔOD_{IS} are the ($OD_{60} - OD_0$) values of the (1) Sample Blank, (2) Sample, and (3) Internal Standard well, respectively. *n* is the sample dilution factor.

For the Standard Curve Method, calculate the L-Arginine concentration of Sample as follows:

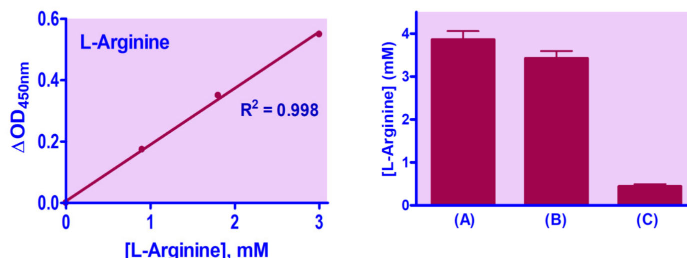
$$[\text{L-Arginine}] = \frac{OD_{SAMPLE} - OD_{H2O}}{\text{Slope } (mM^{-1})} \times n \quad (mM)$$

where OD_{SAMPLE} and OD_{H2O} are the OD values of the Sample and water (Standard #4) at 60 min. Slope is calculated from the standard curve. *n* is the sample dilution factor (if necessary).

Note: if the calculated concentration is higher than 3 mM, dilute sample in water and repeat assay. Multiple the result by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader. NaOH or HCl to neutralize samples.



Left: L-Arginine Standard Curve. *Right*: Juice and human serum samples were prepared according to the protocol. All samples were diluted 5x in dH2O prior to assay. Results: (A) Orange juice: 3.86 ± 0.34 mM; (B) Grape juice: 3.43 ± 0.29 mM; (C) Human serum: 0.44 mM ± 0.07 mM.

LITERATURE

1. Böger, R. H. (2007). The pharmacodynamics of L-arginine. The Journal of Nutrition, 137(6).
2. Verma, N. et al (2017). L-arginine biosensors: A comprehensive review. *Biochemistry and Biophysics Reports*, 12:228–239.
3. Terrade, N., & Mira de Orduna, R. (2006). Impact of winemaking practices on arginine and citrulline metabolism during and after malolactic fermentation. *Journal of Applied Microbiology*, 101(2).

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