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Product Information

L-Arginine Assay Kit

Catalog Number **MAK370**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

L-Arginine (Arg) is a proteogenic, semi-essential amino acid. Healthy humans can synthesize L-arginine using L-glutamine as a building block; however, premature infants are unable to produce Arg and additional supplementation is required for proper growth and development. Arginine plays pivotal roles in biochemical pathways such as the urea cycle and the biosynthesis of nitric oxide. Arginine and ammonia concentrations are elevated in patients having a mutation in their ARG1 genes. The mutation causes lower arginase activities – a condition that is known as argininemia. Arginine has also been advertised as a supplement due to its role in the synthesis of nitric oxide, which helps in vasodilation processes.

The L-Arginine Assay Kit provides a quick, specific, and easy method for the measurement of total L-arginine concentrations in a wide variety of samples. In this enzyme-based assay, L-arginine is converted into a series of intermediates, which will further react with a probe producing a stable colorimetric signal at 450 nm (A_{450}). The kit is simple to use, sensitive and high-throughput adaptable and can detect as low as 1 nmol/well of L-arginine in biological samples.

The kit is suitable for the measurement of L-arginine in biological samples (serum, etc) and beverages (orange juice, etc), the analysis of the relationship of L-arginine intake and nitric oxide production and the analysis of the urea cycle.

Components

The kit is sufficient for 100 colorimetric assays in 96 well plates.

Arginine Assay Buffer Catalog Number MAK370A	25 mL
Arginine Enzyme Mix Catalog Number MAK370B	1 vial
Arginine Probe Mix A Catalog Number MAK370C	12 mL
Arginine Probe Mix B Catalog Number MAK370D	12 mL
Sample Cleanup Mix Catalog Number MAK370E	1 vial
Arginine Standard Catalog Number MAK370F	1 vial

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96 well plates
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)
- Refrigerated microcentrifuge capable of RCF ≥13,000 × *g*

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Preparation Instructions

Reagent Preparation

Arginine Assay Buffer: Warm to room temperature prior to use. May be stored at –20 °C or 2–8 °C.

Arginine Enzyme Mix: Reconstitute vial with 220 µL of Arginine Assay Buffer. Aliquot and store at –20 °C. Keep on ice while in use. Avoid freeze/thaw cycles. Use within two months once reconstituted.

Arginine Probe Mix A and Arginine Probe Mix B: Ready to use as supplied. Warm to room temperature, protected from light, prior to use.

Sample Cleanup Mix: Reconstitute each vial with 220 µL of Arginine Assay Buffer. Aliquot and store at –20 °C. Keep on ice while in use. Avoid freeze/ thaw cycles. Use within two months once reconstituted.

Arginine Standard: Reconstitute with 500 µL of ultrapure water to make a 100 mM stock solution. Store at –20 °C.

Procedure

Sample Preparation

Notes:

- Arginine varies over a wide range for different samples. For unknown samples, perform a pilot experiment with a few dilutions to ensure readings are within the standard curve range. For normal human serum, average arginine concentration is 10–150 µM and can range to 250–1,500 µM for patients with argininemia.
- For samples with arginine concentration close to the detection limit (25 µM), it is recommended to run two samples in parallel and spiking one with a known amount of Arginine Standard (e.g., 4 nmol) to ensure accurate determination of L-arginine.

Biological Fluids: Add 2 µL of sample cleanup mix per 100 µL of sample. Incubate at 37 °C for 1 hour. Centrifuge the treated sample in a Corning Spin-X UF concentrator at 13,000 × *g* for 10 minutes at 4 °C and collect the filtrate.

Beverages: Centrifuge the sample at 13,000 × *g* and discard the precipitate. Collect the supernatant and centrifuge in a Corning Spin-X UF concentrator at 13,000 × *g* for 10 minutes at 4 °C and collect the filtrate.

For all samples, prepare duplicates by adding 2-40 µL of the pretreated, filtered samples in parallel wells. Bring the volume of all wells to 40 µL with Arginine Assay Buffer. Label them as “sample” and “sample background”.

Standard Curve Preparation

Prepare a 4 mM Arginine Standard solution by adding 40 µL of the 100 mM Arginine standard stock to 960 µL of ultrapure water. Prepare Arginie Standards in desired wells of a clear flat-bottom 96 well plate according to Table 1.

Table 1.
Preparation of Arginine Standards

Well	4 mM Premix	Arginine Assay Buffer	Arginine (nmol/well)
1	0 µL	40 µL	0
2	2 µL	38 µL	8
3	4 µL	36 µL	16
4	6 µL	34 µL	24
5	8 µL	32 µL	32
6	10 µL	30 µL	40

Enzyme Mix

Mix enough reagents for the number of assays to be performed. For each well, prepare 10 µL of Enzyme Mix according to Table 2.

Table 2.
Preparation of Enzyme Mix

Reagent	Enzyme Mix	Background Mix
Arginine Enzyme Mix	2 µL	–
Arginine Assay Buffer	8 µL	10 µL

Mix well and add 10 µL of the Enzyme Mix to each well containing standards, samples, and spiked wells (if applicable). Add 10 µL of Background Mix to the sample background wells. Mix well and incubate the plate for 30 minutes at 37 °C.

Reaction Mix

Mix enough reagents for the number of assays to be performed. For each well, prepare 200 µL of Reaction Mix by combining 100 µL of Arginine Probe Mix A plus 100 µL of Arginine Probe Mix B. Mix and add 200 µL of the Reaction Mix to all wells. Mix well and incubate the plate for 60 minutes at 37 °C, protected from light.

Measurement

Measure the absorbance (A_{450}) at 450 nm in a microplate reader in endpoint mode.

Results

1. Subtract the 0 Standard reading from all standard readings.
2. Plot the Arginine Standard Curve.
3. Subtract the Sample background reading from Sample reading ($F_s = A_{450\ sample} - A_{450\ sample\ background}$).
4. For unspiked samples, compare the F_s values to the standard curve to get nmol of L-arginine (B) in the well.

Sample Arginine (nmol/μL or mM) = (B/V) × D

where:
B = Amount of L-arginine, calculated from the standard curve (in nmol)
V = Sample volume added into the reaction well (μL)
D = Sample dilution factor

For spiked L-arginine samples, subtract Sample background from both the Sample reading ($F_s = A_{450\ sample} – A_{450\ sample\ background}$) and the spiked sample reading ($F_{spike} = A_{450\ spike} – A_{450\ sample\ background}$). Calculate amount of L-arginine (B) as follows.

Amount of L-arginine in sample wells (B) =

$$\frac{F_s}{F_{spike} - F_s}\ x\ Arginine\ Spike\ (in\ nmol)$$

Figure 1.
Typical L-Arginine Standard Curve

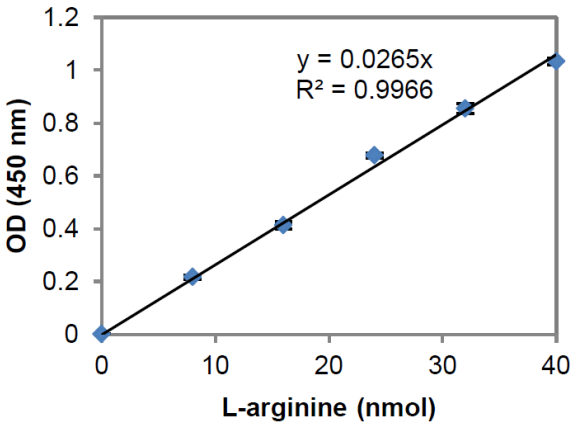
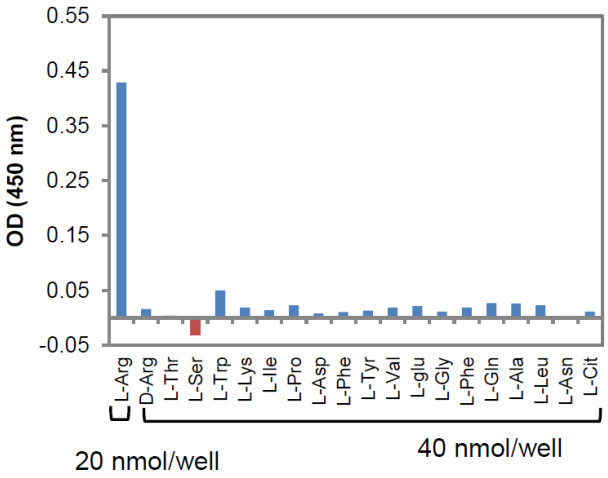
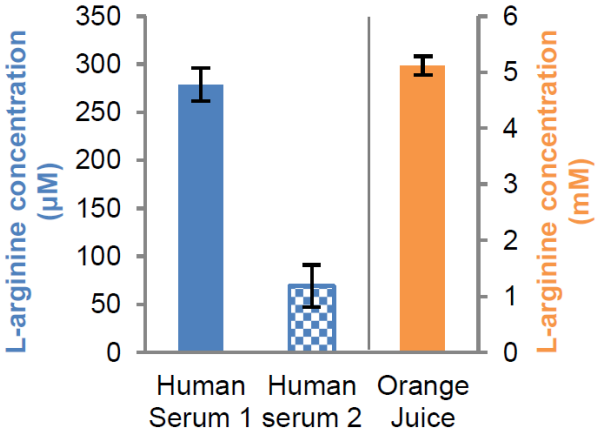


Figure 2.
Specificity of the Detection of L-Arginine over Other Amino Acids



D-arginine and other L-amino acids were tested at a 2-fold molar excess (each amino acid: 40 nmol) vs. L-arginine (20 nmol).

Figure 3.
Estimations of L-Arginine in Two Human Serum samples (30 μL) and Orange Juice (1.6 μL).



L-arginine concentrations were 0.278 mM and 0.069 mM in the two human serum samples, and 5.113 mM in orange juice. Assays were performed following the kit procedure.

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