

## L-Arginine Assay Kit (Colorimetric)

02/19

(Catalog #K749-100; 100 assays; Store at -20°C)

### I. Introduction:

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. BioVision's 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 (OD: 450 nm). 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.



### II. Applications:

- Measurement of L-arginine in biological samples and beverages
- Analysis of relationship of L-arginine intake and nitric oxide production
- Analysis of urea cycle

### III. Sample Type:

- Biological fluids: serum, etc.
- Beverages: orange juice, etc.

### IV. Kit Contents:

| Components            | K749-100 | Cap Code | Part Number |
|-----------------------|----------|----------|-------------|
| Arginine Assay Buffer | 25 ml    | WM       | K749-100-1  |
| Arginine Enzyme Mix   | 1 vial   | Green    | K749-100-2  |
| Arginine Probe Mix A  | 12 ml    | NM/Blue  | K749-100-3  |
| Arginine Probe Mix B  | 12 ml    | NM/Brown | K749-100-4  |
| Sample Cleanup Mix    | 1 vial   | Blue     | K749-100-5  |
| Arginine Standard     | 1 vial   | White    | K749-100-6  |

### V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

### VI. Storage Conditions and Reagent Preparation:

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

- **Arginine Assay Buffer:** Warm to room temperature before use. Store at 4 °C or -20 °C.
- **Arginine Enzyme Mix:** Reconstitute each vial with 220 µl Arginine Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze and thaw. Use within two months.
- **Arginine Probe Mix A, Arginine Probe Mix B:** Ready to use as supplied. Warm to room temperature before use. Store at 4°C or -20°C. Keep away from light.
- **Sample Cleanup Mix:** Reconstitute each vial with 220 µl Arginine Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze and thaw. Use within two months.
- **Arginine Standard:** Reconstitute with 500 µl of dH<sub>2</sub>O to make a 100 mM stock solution. Store at -20°C.

### VII. L-Arginine Assay Protocol:

**1. Sample Preparation: For 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 10kDa MWCO Spin Column (Cat. # 1997) at 13,000 x g for 10 min at 4 °C and collect the filtrate. **For beverages:** Centrifuge the sample at 13,000 x g to discard the precipitate. Collect the supernatant and centrifuge in a 10kDa MWCO Spin Column (Cat. # 1997) at 13,000 x g for 10 min 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".

#### Note:

- Arginine varies over a wide range for different samples. For unknown samples, we recommend performing a pilot experiment with a few dilutions to ensure readings are within the standard curve range. For normal human serum, average arginine concentration 10-150 µM and can range to 250-1500 µM for patients with argininemia.
- For samples with arginine concentration close to the detection limit (25 µM), we recommend running 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.

**2. Standard Curve Preparation:** Prepare a 4 mM solution of Arginine standard by adding 40  $\mu\text{l}$  of the 100 mM Arginine standard stock to 960  $\mu\text{l}$  of  $\text{dH}_2\text{O}$ . Add 0, 2, 4, 6, 8, 10  $\mu\text{l}$  of the 4 mM working Arginine standard into a series of wells, generating 0, 8, 16, 24, 32, 40 nmol of Arginine/well. Adjust the volume to 40  $\mu\text{l}$ /well with Arginine Assay buffer.

**3. Enzyme Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 10  $\mu\text{l}$  Mix containing:

|                       | Reaction Mix    | Background Mix   |
|-----------------------|-----------------|------------------|
| Arginine Enzyme Mix   | 2 $\mu\text{l}$ | --               |
| Arginine Assay Buffer | 8 $\mu\text{l}$ | 10 $\mu\text{l}$ |

Mix well and add 10  $\mu\text{l}$  of the Enzyme Mix to each well containing the Standard, Sample and Spiked wells. Add 10  $\mu\text{l}$  of Background mix to the Sample background wells. Mix well and incubate the plate for 30 min at **37°C**.

**4. Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 200  $\mu\text{l}$  Mix containing:

|                      |                   |
|----------------------|-------------------|
| Arginine Probe Mix A | 100 $\mu\text{l}$ |
| Arginine Probe Mix B | 100 $\mu\text{l}$ |

Mix and add 200  $\mu\text{l}$  of the Reaction Mix to each well containing the Standard, Sample, Sample Background and Spiked wells. Mix well and incubate the plate for 60 min at **37 °C**. Protect from light.

**5. Measurement:** Measure absorbance (OD: 450 nm) in a microplate reader in endpoint mode.

**6. Calculation:** Subtract 0 Standard reading from all standard readings. Plot the arginine Standard Curve. Subtract Sample background reading from Sample reading ( $F_s = \text{OD}_{\text{sample}} - \text{OD}_{\text{sbc}}$ ). For unspiked samples, apply the  $F_s$  values to the standard curve to get B nmol of L-arginine in the well.

$$\text{Sample L-arginine Concentration} = \frac{B}{V} \times D = \text{nmol}/\mu\text{l} = \text{mM}$$

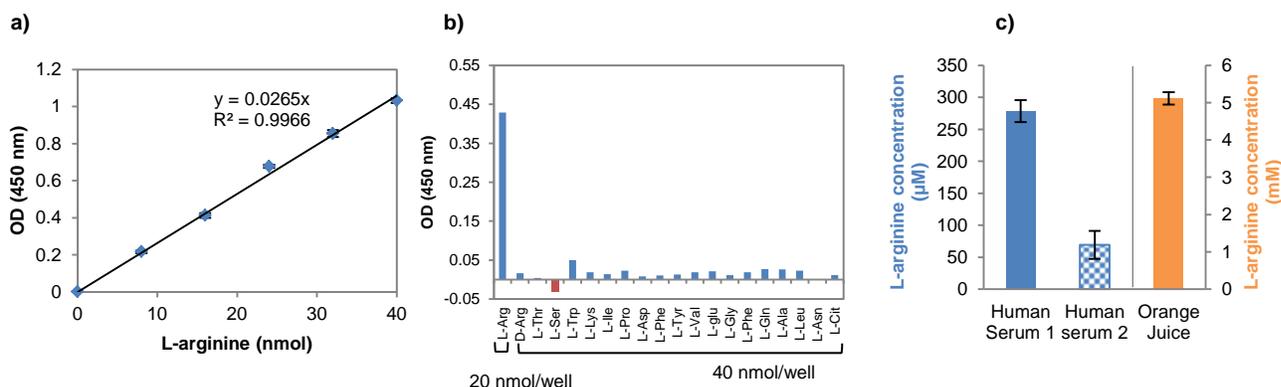
**Where:** B is the amount of L-arginine, calculated from the standard curve (in nmol)

V is the volume of sample added to the well (in  $\mu\text{l}$ )

D is the sample dilution factor (if applicable, D=1 for undiluted samples)

**Note:** For spiked L-arginine samples, subtract Sample background from both of the Sample reading ( $F_s = \text{OD}_{\text{sample}} - \text{OD}_{\text{sbc}}$ ) and the Spiked sample reading and Spike reading ( $F_{\text{spike}} = \text{OD}_{\text{spike}} - \text{OD}_{\text{sbc}}$ ). Calculate amount of L-arginine (B) as follows.

$$\text{Amount of L-arginine in sample wells (B)} = \frac{F_s}{F_{\text{spike}} - F_s} \times \text{Arginine Spike (in nmol)}$$



**Figure:** (a) L-arginine standard curve; (b) 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 AA: 40 nmol) vs L-arginine (20 nmol). (c) Estimations of L-arginine in two human serum sample (30  $\mu\text{l}$ ) and orange juice (1.6  $\mu\text{l}$ ). 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 protocol.

#### VIII. RELATED PRODUCTS:

DL-serine Assay Kit (Fluorometric) (K545)

Alanine Colorimetric/Fluorometric Assay Kit (K652)

Glutamine Assay Kit (K556)

Glutamate Assay Kit (K629)

Aspartate Assay Kit (K552)

Total D-amino acid Assay Kit (Fluorometric) (K445)

Glycine Assay Kit (K589)

Cysteine Assay Kit (K558)

Tyrosine Assay Kit (K573)

Phenylalanine Assay Kit (K572)

**FOR RESEARCH USE ONLY! Not to be used on humans.**