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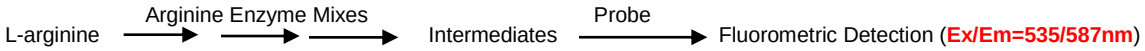
Arginine Assay Kit (Fluorometric)

12/18

(Catalog #K384-100; 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 our enzyme-based assay, L-arginine is converted into a series of intermediates, which will further react with a probe producing a stable fluorescence signal (Ex/Em = 535/587 nm). The kit is simple to perform, specific, high-throughput adaptable and sensitive– it can detect as low as 100 pmol/well of L-arginine in human biofluids and other biological samples.



II. Applications:

- Measurement of Arginine in biological samples
- Analysis of urea cycle

III. Sample Type:

- Biological fluids: serum, etc.
- Animal tissues

IV. Kit Contents:

Components	K384-100	Cap Code	Part Number
Arginine Assay Buffer	25 ml	WM	K384-100-1
Arginine Enzyme Mix	1 vial	Blue	K384-100-2
Arginine Developer Mix	1 vial	Orange	K384-100-3
Arginine Converter Mix	1 vial	Purple	K384-100-4
Arginine Signal Mix	1 vial	Green	K384-100-5
Arginine Probe (in DMSO)	200 µl	Red	K384-100-6
Arginine Standard	1 vial	White	K384-100-7

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Dounce Tissue Homogenizer (Cat. #1998)
- 1 M Dithiothreitol (DTT) solution
- 50% glycerol solution

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 ºC or -20 ºC.
- **Arginine Enzyme Mix, Arginine Converter Mix, Arginine Signal 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 Developer Mix:** ***Only reconstitute prior to use!*** Mix 5 µl of 1 M DTT solution with 995 µl of 50% glycerol solution. Add 40 µl of the DTT/50% glycerol solution into the vial. Vortex for 5 seconds. Incubate at 25 ºC for 30 minutes. Completely dissolved Arginine Developer Mix should be a viscous clear yellow solution. Aliquot and store at **-80°C**. Avoid freeze and thaw. Use within two months.
- **Arginine Probe (in DMSO):** Ready to use as supplied. Warm to room temperature before use. Store at 4ºC or -20°C. Protect from light.
- **Arginine Standard:** Reconstitute with 500 µl of dH_2O to make a 100 mM stock solution. Store at -20°C.

VII. Arginine Assay Protocol:

1. **Sample Preparation: For tissue samples:** Rapidly homogenize tissue (~10 mg) in 100 µl ice cold Arginine Assay Buffer with Dounce Tissue Homogenizer (Cat. #1998), and keep on ice for 10 min. Centrifuge at 10,000 x *g* for 10 min at 4 °C. Carefully transfer the supernatant to a 10 kDa MWCO Spin Column (Cat. # 1997). Centrifuge the sample at 10,000 *x g* for 20 min at 4 °C and collect the filtrate. **For biological fluids:** Centrifuge at 10,000 x *g* for 10 min at 4 °C to remove any insoluble precipitate in the biological fluids. Add 200-500 µl of sample into a 10 kDa MWCO Spin Columns (Cat. # 1997). Centrifuge the sample at 10,000 *x g* for 20 min at 4 °C and collect the filtrate. **For all samples:** Due to matrix effect in biological samples, an internal standard (Spike) is needed for each sample. For each test sample, prepare 3 parallel sample wells. Add 2-50 µl of samples into 3 wells in a 96-well black plate. Label each well as "Sample", "Sample background", "Spike". Dilute Arginine standard to 1 mM by adding 10 µl of the 100 mM stock solution into 990 µl of dH_2O. Prepare a 0.1 mM solution by adding 10 µl of the 1 mM stock into 90 µl of dH_2O. Add 4 µl of the 0.1 mM arginine standard into

the "spike" wells. Bring the volume of all wells to 50 µl with Arginine Assay buffer. Prepare 2 wells with 50 µl Arginine Assay Buffer labeled as "Blank" and "Reagent Control". For unknown samples, prepare wells with different dilutions.

2. **Standard Curve Preparation (Optional):** Prepare a 1 mM solution of Arginine standard by adding 10 µl of the 100 mM Arginine standard stock to 990 µl of dH_2O. Prepare a 0.1 mM solution by adding 10 µl of the 1 mM stock into 90 µl of dH_2O. Add 0, 4, 8, 12, 16, 20 µl of the 0.1 mM working Arginine standard into a series of wells, generating 0, 400, 800, 1200, 1600, 2000 pmol of Arginine/well. Adjust the volume to 50 µl/well with the Arginine Assay buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. Prepare a 50-fold dilution of the Arginine Developer Mix (e.g. Mix 4 µl of Arginine Developer Mix with 196 µl Arginine Assay Buffer. Note *50% glycerol solution is viscous. Handle Arginine Developer solution carefully*. Prepare a 5-fold dilution of Arginine Probe (e.g. Mix 4 µl of Arginine Probe with 20 µl Arginine Assay Buffer). For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Mix
Arginine Assay Buffer	39 µl	41 µl
Arginine Enzyme Mix	2 µl	----
Diluted Arginine Developer Mix	3 µl	3 µl
Arginine Converter Mix	2 µl	2 µl
Arginine Signal Mix	2 µl	2 µl
Diluted Arginine Probe	2 µl	2 µl

Mix and add 50 µl of the Reaction Mix to each well containing the Blank, Standard, Sample and Spike wells. Add 50 µl of the background Mix into Sample Background and Reagent Control. Mix well and incubate the plate for 60 min at **37 °C**. Protect from light. ***Do not store diluted working solutions.***

4. **Measurement:** Measure fluorescence (Ex/Em= 535/587nm) in a microplate reader in endpoint mode.
5. **Calculation:** Subtract 0 standard readings from all standard readings. For reference, plot the Arginine Standard Curve. Subtract Reagent Control readings from Blank ($F_{corrected}$ = RFU_{blank} – RFU_{RC}). Subtract sample backgrounds reading from sample (F_s = RFU_s - RFU_{sbc}) and spike readings (F_{spike} = RFU_{spike} – RFU_{sbc}).

Amount of Arginine in sample wells (B) = $\frac{F_s - F_{corrected}}{F_{spike} - F_s} \times 400$ pmol

For biological fluids: Sample Arginine Concentration = $\frac{B}{V} \times D$ = pmol/ µl = µM

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

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

For tissue samples: Sample Arginine Concentration = $\frac{B}{V \times P} \times D$= pmol/ µg

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

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

P is the sample protein concentration in the untreated samples (µg-protein/µl)

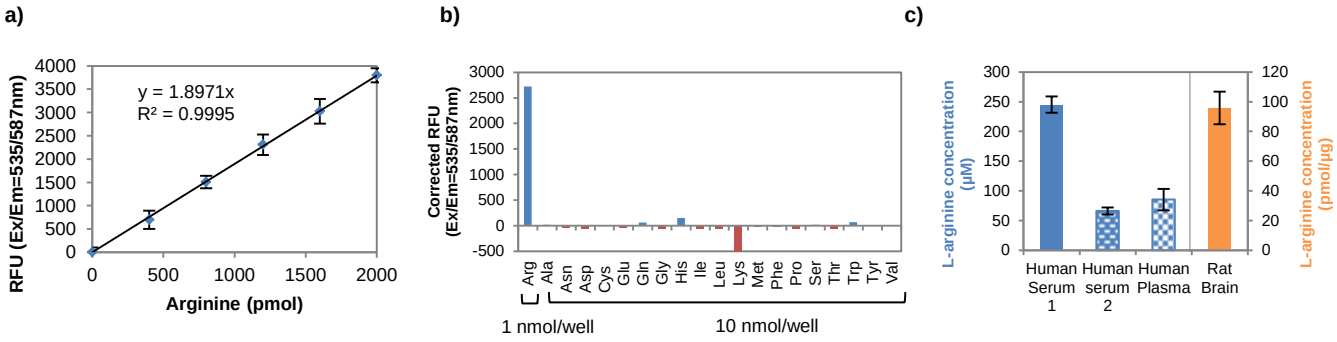


Figure: (a) Arginine standard curve; (b) Specificity of the detection of arginine over other amino acids: Other amino acids were tested at a 10-fold molar excess (each AA: 10 nmol) vs Arginine (1 nmol). (c) Estimations of Arginine in human serum and plasma sample (4 µl each well) and rat brain (5.5 µg protein). L-arginine concentrations were 245 and 66.1 µM in human serum samples, 85.3 µM in human plasma, and 95.8 pmol/µg-protein in rat brain. Assays were performed following the kit protocols.

VIII. RELATED PRODUCTS:

DL-serine Kit (K545)	Glycine Kit (K589)	Glutamate Kit (K629)	Total D-amino acid Kit (K445)
Alanine Kit (K652)	Phenylalanine Kit (K572)	Glutamine Assay Kit (K556)	Arginine (Colorimetric) Kit (K749)
Cysteine Kit (K558)	Tyrosine Kit (K573)	Aspartate Kit (K552)	Ornithine Kit (Kxxx)

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