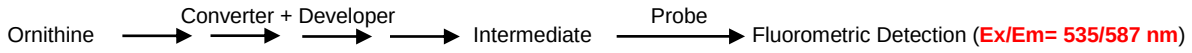
Ornithine Assay Kit (Fluorometric)

12/18

(Catalog #K939-100; 100 assays; Store at -20°C)

I. Introduction:

Ornithine is a non-standard, non-proteogenic amino acid. Ornithine is converted to putrescine via ornithine decarboxylase, which is the first step in the synthesis of polyamines. Additionally, Ornithine is one of the main metabolites that participate in the urea cycle in order to excrete excess nitrogen in the form of urea. In cells, Arginase hydrolyzes Arginine producing Ornithine and Urea (cytosol). Then, ornithine enters the mitochondria, reacts with carbamoyl phosphate forming Citrulline. Ornithine has therapeutics uses: it is administered to patients suffering brain complications caused by hepatic encephalopathy. Additionally, early research has suggested Ornithine may improve performance in some professional athletes. BioVision's Ornithine Assay Kit provides a rapid, specific, and easy method for the measurement of total ornithine concentrations in a wide variety of biological samples. In this enzyme-based assay, ornithine is converted into a series of intermediates, which will further react with a probe producing a stable fluorometric signal (Ex/Em = 535/587nm). The kit is simple to use, sensitive and high-throughput adaptable and can detect as low as 50 pmol/well of ornithine in biological samples.



II. Applications:

- Measurement of Ornithine in biological samples
- Analysis of urea cycle and synthesis of polyamines

III. Sample Type:

- Biological fluids: serum, etc.
- Animal tissues

IV. Kit Contents:

Components	K939-100	Cap Code	Part Number
Ornithine Assay Buffer	25 ml	WM	K939-100-1
Ornithine Converter Mix	1 vial	Orange	K939-100-2
Ornithine Developer Mix	1 vial	Purple	K939-100-3
Ornithine Enzyme Mix	1 vial	Green	K939-100-4
Ornithine Probe (in DMSO)	200 µl	Red	K939-100-5
Tissue Cleanup Mix	1 vial	Blue	K939-100-6
Ornithine Standard	1 vial	Yellow	K939-100-7

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.

- **Ornithine Assay Buffer:** Warm to room temperature before use. Store at 4 ºC or -20 ºC.
- **Ornithine Converter 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 Ornithine Converter Mix should be a viscous clear yellow solution. Aliquot and store at **-80°C**. Avoid freeze and thaw. Use within two months.
- **Ornithine Developer Mix, Ornithine Enzyme Mix:** Reconstitute each vial with 220 µl Ornithine Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze and thaw. Use within two months.
- **Ornithine Probe (in DMSO):** Ready to use as supplied. Warm to room temperature before use. Store at 4ºC or -20°C. Keep away from light.
- **Tissue Cleanup Mix:** Reconstitute each vial with 220 µl Ornithine Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze and thaw. Use within two months.
- **Ornithine Standard:** Reconstitute with 100 µl of dH_2O to make a 100 mM stock solution. Store at -20°C.

VII. Ornithine Assay Protocol:

1. **Sample Preparation: For tissue samples:** Rapidly homogenize tissue (~10 mg) in 100 µl ice cold Ornithine 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 1.5 ml microcentrifuge tube. Add 2 µl of the Sample CleanUp Mix into 100 µl of the tissue lysate. Incubate at 37 °C for 30 min. Transfer the treated samples into a 10kDa 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 10kDa 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 (spiking) is needed for each sample. For each test sample, prepare 3 parallel sample wells. Add 2-50 µl of samples (2-10 µl of rat liver and human serum) into 3 wells in a 96-well black plate. Label each well as "Sample", "Sample background", "Spike". Dilute Ornithine standard to 1

mM by adding 10 µl of the 100 mM stock solution into 990 µl of dH_2O. Further dilute the 1 mM standard solution into 0.1 mM standard solution by adding 10 µl of the 1mM stock into 90 µl of dH_2O. Add 4 µl of the 0.1 mM ornithine standard into the wells designated as "spike". Bring the volume of all the wells to 50 µl with Ornithine Assay buffer. Prepare 2 wells with 50 µl Ornithine Assay Buffer labeled as "Blank" and "Reagent Control". For unknown samples, prepare parallel wells with different dilutions.

2. **Standard Curve Preparation (Optional):** Prepare a 1 mM solution of Ornithine standard by adding 10 µl of the 100 mM Ornithine standard stock to 990 µl of dH_2O. Further dilute the 1 mM solution into a 0.1 mM solution by adding 10 µl of the 1 mM Ornithine standard solution into 90 µl of dH_2O. Add 0, 2, 4, 6, 8, 10 µl of the 0.1 mM working Ornithine standard into a series of wells, generating 0, 200, 400, 600, 800, 1000 pmol of Ornithine/well. Adjust the volume to 50 µl/well with the Ornithine Assay buffer. *Do not store diluted standards.*
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. Prepare a 100-fold dilution of the Ornithine Converter Mix (e.g. Mix 2 µl of Ornithine Converter Mix with 198 µl Ornithine Assay Buffer. *50% glycerol solution is viscous. Handle Ornithine Converter solution carefully*. Prepare a 5-fold dilution of Ornithine Probe (e.g. Mix 5 µl of Ornithine Probe with 20 µl Ornithine Assay Buffer). For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Mix
Ornithine Assay Buffer	38 µl	44 µl
Diluted Ornithine Converter Mix	6 µl	----
Ornithine Enzyme Mix	2 µl	2 µl
Ornithine Developer Mix	2 µl	2 µl
Diluted Ornithine 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 wells and Reagent Control. Mix well and incubate the plate for 30 min at **37 °C**. Protect from light.

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 Ornithine 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 Ornithine in sample wells (B) = $\frac{F_s - F_{corrected}}{F_{spike} - F_s} \times$ Ornithine Spike (in pmol)

For biological fluids: Sample Ornithine 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 Ornithine Concentration= (B * D) / (V * P) (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)

a) **b)** **c)**

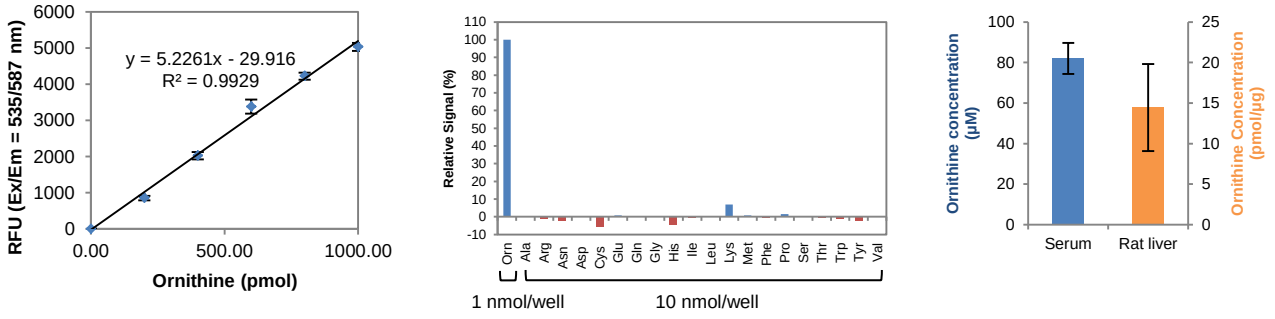


Figure: (a) Ornithine standard curve; (b) Specificity of the detection of Ornithine over other amino acids: Other L-amino acids were tested at a 10-fold molar excess (each AA: 10 nmol) vs Ornithine (1 nmol). (c) Estimations of Ornithine in human serum sample (10 µl) and rat liver (62 µg protein). Ornithine concentrations were 82.1 µM in human serum sample, and 14.5 pmol/µg-protein in rat liver. Assays were performed following the kit protocol.

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)	Total Polyamine Kit (K475)

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