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ab252903 Ornithine Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab252903
(use https://www.abcam.cn/ab252903 for china, or
https://www.abcam.co.jp/ab252903 for Japan)

For the measurement of Ornithine in biological samples.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

The Ornithine Assay Kit (Fluorometric) (ab252903) 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.

2. Protocol Summary

Prepare all samples, standards and controls as instructed.

Add 2-50 µL of each sample into a 96 well clear plate; adjust final volume to 50 µL with Assay Buffer 65.

Create the 50 µL Reaction Mix and add to standards, blank, spike wells and test samples.

Create the background mix and add 50 µL into the Sample Background and reagent control wells.

Mix well and incubate the plate for 30 minutes at 37°C protected from light.

Measure fluorescence (Ex/Em = 535/587nm) in a microplate reader in endpoint mode.

3. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer 65	25 mL	-20°C	-20°C
Ornithine Converter Mix	1 vial	-20°C	-20°C
Developer Solution V	1 vial	-20°C	-20°C
Converter Mix K	1 vial	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
Sample Clean-Up Mix I	1 vial	-20°C	-20°C
Ornithine Standard	1 vial	-20°C	-20°C

PLEASE NOTE: Assay Buffer 65 was previously labelled as Ornithine Assay Buffer and Assay Buffer LXV, and Developer Solution V as Ornithine Developer Mix, and Converter Mix K as Converter Enzyme XIII and Ornithine Enzyme Mix, and OxiRed™ Probe as Ornithine Probe (in DMSO), and Sample Clean-Up Mix I as Tissue Cleanup Mix. The composition has not changed.

4. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

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

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 Assay Buffer 65:

Warm to room temperature before use. Store at -20°C.

6.2 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 °C for 30 minutes. Completely dissolved Ornithine Converter Mix should be a viscous clear yellow solution. Aliquot and store at -20°C. Avoid freeze/thaw cycle. Use within two months.

6.3 Developer Solution V:

Reconstitute each vial with 220 µL Assay Buffer 65. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze/thaw cycle. Use within two months.

6.4 Converter Mix K:

Reconstitute each vial with 220 µL Assay Buffer 65. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze/thaw cycle. Use within two months.

OxiRed™ Probe:

Ready to use as supplied. Warm to room temperature before use. Store at -20°C. Keep away from light.

6.5 Sample Clean-Up Mix I:

Reconstitute each vial with 220 µL Assay Buffer 65. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze thaw cycle. Use within two months.

6.6 Ornithine Standard:

Reconstitute with 100 µL of dH_2O to make a 100 mM stock solution. Store at -20°C.

7. Sample preparation

7.1 For whole cells or tissue:

- Rapidly homogenize tissue (~10 mg) in 100 µL ice cold Assay Buffer 65 with Dounce Tissue Homogenizer, and keep on ice for 10 minutes.
- 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 Clean-Up Mix I into 100 µL of the tissue lysate.
- Incubate at 37 °C for 30 min. Transfer the treated samples into a 10kDa MWCO Spin Column. Centrifuge the sample at 10,000 x g for 20 min at 4 °C and collect the filtrate.

7.2 For Biological fluids:

- Centrifuge at 10,000 x *g* for 10 minutes at 4 °C to remove any insoluble precipitate in the biological fluids. Add 200-500 µL of sample into a 10kDa MWCO Spin Columns. Centrifuge the sample at 10,000 x *g* for 20 minutes at 4 °C and collect the filtrate.

7.3 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 Assay Buffer 65.
- Prepare 2 wells with 50 µL Assay Buffer 65 labeled as "Blank" and "Reagent Control". For unknown samples, prepare parallel wells with different dilutions.

8. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

8.1 Prepare a 1mM solution of Orthinine Standard by adding 10 µL of the 100 mM Orthinine standard stock to 990 µL of dH_20.

8.2 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.

8.3 Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 mM working Ornithine standard solution to a series of wells in duplicate in a 96-well plate.

8.4 Bring the total volume of each well to 50 µL with Assay Buffer 65 to generate 0, 200, 400, 600, 800, and 1000 pmol of Ornithine per well.

Standard#	Ornithine Standard (µL)	Assay Buffer 65 (µL)	End amount of Ornithine in well (pmol/well)
1	0	50	0
2	2	48	200
3	4	46	400
4	6	44	600
5	8	42	800
6	10	40	1000

9. Assay Procedure

9.1 Standard Curve Preparation (Optional):

1. Prepare a 1 mM solution of Ornithine standard by adding 10 µl of the 100 mM Ornithine standard stock to 990 µl of dH2O. 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 dH2O. 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 Assay Buffer 65. Do not store diluted standards.

9.2 Reaction mix:

1. Mix enough reagents for the number of assays to be performed.
2. Prepare a 100-fold dilution of the Ornithine Converter Mix (e.g. Mix 2 µL of Ornithine Converter Mix with 198 µL Assay Buffer 65. **ΔNote:** 50% glycerol solution is viscous. Handle Ornithine Converter solution carefully.
3. Prepare a 5-fold dilution of OxiRed™ Probe (e.g. Mix 5 µL of OxiRed™ Probe with 20 µL Assay Buffer 65).
4. For each well, prepare the 50µL Mix shown Below:

	Reaction Mix	Background Control Mix
Assay Buffer 65	38 µL	44 µL
Diluted Ornithine Converter Mix	6 µL	-
Converter Mix K	2 µL	2 µL
Developer Solution V	2 µL	2 µL
OxiRed™ Probe	2 µL	2 µL

5. Mix and add 50 µL of the Reaction Mix to each well containing the Blank, Standard, Sample and Spike wells.
6. Add 50 µL of the background Mix into Sample Background wells and Reagent Control.
7. Mix well and incubate the plate for 30 minutes at 37°C. Protected from the light.

9.3 Measurement:

1. Measure fluorescence (Ex/Em = 535/587nm) in a microplate reader in endpoint mode.

9.4 Calculation:

1. Subtract the 0 standard reading from all standard readings. For reference, plot the Ornithine standard curve.
2. Subtract Reagent Control readings from blank: ($F_{corrected}$ = RFU_{blank} – RFU_{RC}).
3. 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**):

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

For Biological fluids:

Sample Ornithine concentration = $\frac{B}{V} \times D$ = pmol/µL = µM

Where:

V = Sample volume added into the reaction well (in µL)

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

For Tissue sample:

Sample Ornithine Concentration $= \frac{(B \times D)}{(V \times P)}$ = pmol/µg

Where:

V = Sample volume added into the reaction well (in µL)

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

P = The sample protein concentration in the untreated samples (µg-protein/µL).

10. Typical Data

Data provided **for demonstration purposes only.**

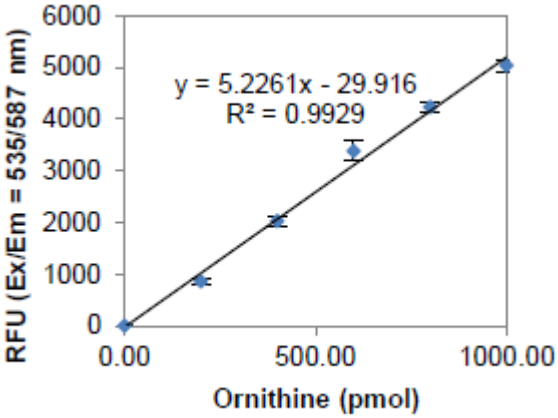

Figure 1. Ornithine Standard curve for reference only.

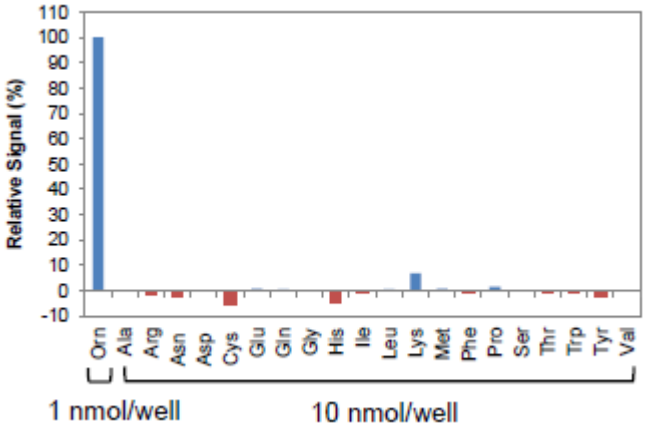

Figure 2. Specificity of the detection of Ornithine over other amino acids: Other L-amino acids were tested at a 10-fold molar excess (each AA: 10 nmol) vs Ornithine (1 nmol).

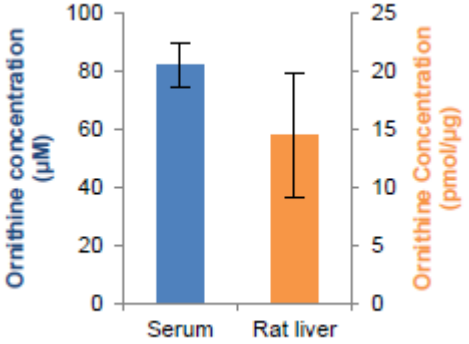

Figure 3. Estimations of Ornithine in human serum sample (10 µL) and rat liver (62 µg protein). Ornithine concentrations were 82.1 µM in human serum sample, and 14.5 pmol/µg-protein in rat liver. Assays were performed following the kit protocol.

11. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)