ab252903 Ornithine Assay Kit (Fluorometric)

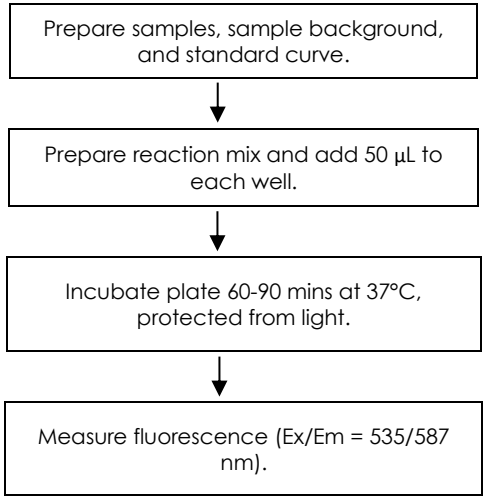
For rapid, sensitive and accurate measurement of Ornithine in biological samples.
For research use only - not intended for diagnostic use.

Background:

Ornithine Assay Kit (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 = 535/587 nm). The kit is simple to use, sensitive, high-throughput adaptable, and can detect as low as 50 pmoles/well of ornithine in biological samples.

Assay Summary:

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.



QUICK ASSAY PROCEDURE

- Thaw and/or reconstitute reagents where applicable and prepare equipment.
- Prepare samples in duplicate.
- Prepare standard curve and make dilutions to Ornithine Converter Mix and OxiRed™ Probe.
- Set up plate for standards, samples, and background controls.
- Prepare Reaction Mix and Background Control Mix.
- Add 50 µL Reaction Mix to each Standard, Sample, Spike, and Blank wells.
- Add 50 µL of Background Control Mix to Background and Reagent Control wells.
- Incubate plate at 37°C for 60-90 minutes, then measure fluorescence (Ex/Em 535/587 nm) in endpoint mode.
- Alternatively, immediately measure fluorescence (Ex/Em=535/587 nm) in kinetic mode for 60-90 minutes at 37°C directly after adding Reaction Mixes.

Precautions & Limitations:

Please read these instructions carefully prior to beginning the assay.
All kit components have been formulated and quality control tested to function successfully as a kit.

- Modifications to the kit components or procedures may result in loss of performance.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

Storage and Stability:

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Ornithine Assay Buffer	25 mL	-20°C	+4°C to -20°C
OxiRed™ Probe	200 µL	-20°C	-20°C
Converter Mix K	1 vial	-20°C	-20°C
Ornithine Converter Mix	1 vial	-20°C	-20°C
Developer Solution V	1 vial	-20°C	-20°C
Sample Clean-Up Mix I	1 vial	-20°C	-20°C
Ornithine Standard	1 vial	-20°C	-20°C

Note: Converter Mix K was previously labelled as Converter Enzyme XIII or Ornithine Enzyme Mix. OxiRed™ Probe was previously labelled as Ornithine Probe (in DMSO). Developer Solution V was previously labelled as Ornithine Developer Mix, and Sample Clean-Up Mix I as Tissue Cleanup Mix. The component names have changed, but the kit mechanism of detection remains unchanged.

Materials Required, Not Supplied:

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm
- 96 well black plate with flat bottom
- Microcentrifuge
- Dounce homogenizer (tissue samples)
- 10 kDa MWCO spin column
- 1 M Dithiothreitol (DTT) Solution
- 50% glycerol solution
- MilliQ water or other type of double distilled/deionized water (ddH2O)

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

Ornithine Assay Buffer: Allow to warm to room temperature (RT) before use. Keep away from light. Store at +4°C to -20°C.

OxiRed™ Probe: Provided as a solution in DMSO. Divide into aliquots and store at -20°C, protected from light. Prior to use, warm solution to room temperature and ensure all stock is thawed. After use, promptly retighten cap to minimize absorption of airborne moisture.

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/glycerol solution into the component vial. Vortex for 5-10 seconds and incubate at 25°C for 30 minutes. Completely dissolved Ornithine Converter Mix should be a viscous, clear-yellow solution. Aliquot and store at **-80°C** for up to two months, avoid repeated freeze/thaws.

Converter Mix K: Reconstitute with 220 µL Ornithine Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use and use the reconstituted stock within two months.

Developer Solution V: Reconstitute with 220 µL Ornithine Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use and use the reconstituted stock within two months.

Sample Clean-Up Mix I: Reconstitute with 220 µL Ornithine Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use and use the reconstituted stock within two months.

Ornithine Standard: Reconstitute with 1000 µL of ddH_2O and mix thoroughly to generate a 100 mM Ornithine Standard solution. Aliquot and store at -20°C. Keep on ice while in use and use the reconstituted stock within two months.

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- Each dilution has enough standard to set up duplicate readings (2 x 50 µL).
- If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.

Prepare Ornithine Standard as follows:

1. Prepare a 1 mM solution of Ornithine Standard by adding 10 µL of the 100 mM Ornithine Standard stock to 990 µL of ddH_2O.
2. Further dilute the 1 mM solution to 0.1 mM by adding 10 µL of the 1 mM solution into 90 µL of ddH_2O.
3. Add 0, 2, 4, 6, and 10 µL of the 0.1 mM Ornithine Standard solution into a series of wells in a black 96-well plate to generate 0, 200, 400, 600, 800, and 1000 pmol/well of Ornithine. Bring the total volume of each well to 50 µL with Ornithine Assay Buffer.
4. Do not store the diluted standards.

Standard #	Volume of 0.1 mM Standard (µL)	Assay Buffer (µL)	Ornithine Standard/well (pmol)
1	10	40	1000
2	8	42	800
3	6	44	600
4	4	46	400
5	2	48	200
6	0	50	0

Sample Preparation:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples, and the readings can be lower than expected.

Whole cells or tissue samples:

1. Rapidly homogenize tissue (~10 mg) in 100 µL of ice-cold Ornithine Assay Buffer with a Dounce Tissue Homogenizer and keep on ice for 10 minutes.
2. Centrifuge at 10,000 x *g* for 10 minutes at 4°C. Carefully transfer the supernatant to a 1.5 mL microcentrifuge tube.
3. Add 2 µL of the Sample Clean-Up Mix I into 100 µL of the tissue lysate.
4. Incubate at 37°C for 30 minutes. Transfer the treated samples into a 10 kDa MCWO spin column and centrifuge the sample at 10,000 x *g* for 20 minutes at 4°C and collect the filtrate.

Biological Fluids:

1. Centrifuge at 10,000 x *g* for 10 minutes at 4°C to remove any insoluble precipitate in the biological fluid. Add 200-500 µL of the sample to a 10 kDa MCWO spin column and centrifuge at 10,000 x *g* for 20 minutes at 4°C and collect the filtrate.

For All Samples:

Due to potential matrix effects in different types of biological samples, an internal standard (spiking) is needed for each sample. The use of this single-point standard addition (spiking) method (in which unspiked and spiked test sample wells are assayed in parallel) is required to ensure accurate quantification.

1. For each test sample, prepare 3 parallel sample well. Add 2-50 µL of samples (for serum, 2-10 µL is recommended) into 3 wells of a 96-well black flat-bottom plate.
2. Label wells for "sample", "sample background", and "spike". Dilute the Ornithine Standard to 1 mM by adding 10 µL of the 100 mM standard stock into 990 µL of ddH_2O.
3. Further dilute the 1 mM standard solution to 0.1 mM solution by adding 10 µL of the 1 mM solution into 90 µL of ddH_2O.
4. Add 4 µL of the 0.1 mM Ornithine Standard solution into the "spike" wells. Bring the volume of all wells to 50 µL with Ornithine Assay Buffer.
5. Prepare 2 wells with 50 µL of Ornithine Assay Buffer for "blank" and "reagent control" wells. For unknown samples, prepare parallel wells with different dilutions.

Note: Once treated with Sample Clean-Up Mix I and filtered, cell and tissue lysates can be stored at -80°C.

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay.
- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate.
- Mix enough reagents for the number of assays to be performed.

1. Prepare a 100-fold dilution of the Ornithine Converter Mix (i.e. Mix 2 µL of the Ornithine Converter Mix with 198 µL of Ornithine Assay Buffer).
2. Prepare a 5-fold dilution of OxiRed™ Probe (i.e. Mix 5 µL of OxiRed™ Probe with 20 µL of Ornithine Assay Buffer).
3. Prepare 50 µL Reaction Mix for each well to be assayed as per the table below and mix well. Add 50 µL of Reaction Mix into Standard, Sample, Blank, and Spike wells.
4. For Sample Background wells, mix and add 50 µL of the Sample Background Mix to each Sample Background and Reagent Control wells.

Component	Reaction Mix (µL)	Background Mix (µL)
Ornithine Assay Buffer	38	44
Diluted Ornithine Converter Mix	6	0
Converter Mix K	2	2
Developer Solution V	2	2
Diluted OxiRed™ Probe	2	2

5. Incubate at 37°C for 60-90 minutes protected from light. After incubation, measure fluorescence (Ex/Em = 535/587 nm) in endpoint mode.
6. Alternatively, immediately read plate after Reaction Mixes have been added at fluorescence (Ex/Em = 535/587 nm) at 37°C in kinetic mode for 60-90 minutes.

Calculations:

- For samples producing signals greater than that of the highest standard: dilute further in appropriate buffer and reanalyze. Multiply the concentration found by the appropriate dilution factor.

1. Subtract 0 pmole Ornithine Standard reading from all standard curve readings and plot the background-subtracted Ornithine Standard curve.
2. Draw the best smooth curve through these points to construct the standard curve. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
3. Subtract sample background reading from samples (F_s= RFU_s – RFU_{sbc}) and spike readings (F_{spike} = RFU_{spike} - RFU_{sbc}).

Amount Ornithine in sample well (**B**):

$$B = \left(\frac{F_S - F_{corrected}}{F_{spike} - F_S}\right) x\ Ornithine\ Spike\ (pmole)$$

For biological fluids:

$$Sample\ Ornithine\ Concentration = \left(\frac{B}{V}\right) x\ D = \text{pmole/µL} = \text{µM}$$

Where:
B = amount of Ornithine in the sample
V = sample volume added to the reaction well (µL)
D = sample dilution factor (if applicable, D=1 for undiluted samples)

For tissue samples:

$$Sample\ Ornithine\ Concentration = \frac{(B\ x\ D)}{(V\ x\ P)} = pmole/\mu g$$

Where:
V= Sample volume added to reaction well (µL)
D= Sample dilution factor (if applicable, D=1 for undiluted samples)
P= Sample protein concentration in the untreated samples (µg protein / µL)

Technical Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

For technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
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