Oxygen Consumption Rate (OCR) Assay Kit

Cat #: KTA9010 Size: 48 T/96 T

	Oxygen Consumption Rate (OCR) Assay Kit		
REF	**Cat #:** KTA9010	LOT	**Lot #:** Refer to product label
	Applications: Fluorescence Microplate Reader		
	Fluorescence Excitation/Emission: OCR-Probe: Ex/Em= 405/650 nm,		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Cellular oxygen consumption mainly derives from mitochondrial oxidative phosphorylation, a process driving ATP production. The cellular Oxygen Consumption Rate (OCR) serves as a critical readout for evaluating mitochondrial function. Research shows tumor cells preferentially generate ATP via glycolysis, a low-efficiency energy pathway; immune cells exhibit suppressed anti-tumor capacity when relying primarily on oxidative phosphorylation, while enhanced glycolysis boosts their anti-tumor effects. Therefore, OCR is widely used to characterize cellular metabolic phenotypes.

The probe included in this kit undergoes phosphorescence quenching upon binding oxygen, with signal intensity negatively correlated to dissolved oxygen concentration in culture medium. Sustained oxygen consumption via mitochondrial respiration reduces extracellular dissolved oxygen, alleviating probe quenching and gradually elevating the probe's fluorescent signal as oxygen is depleted.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
OCR-Probe	0.5 mL	1 mL	4℃, protected from light
Mineral Oil	10 mL	10 mL×2	4℃
10 mM CCCP	0.25 mL	0.5 mL	4℃, protected from light
100 U/mL GOD	0.5 mL	1 mL	4℃

Materials Required but Not Supplied

·Fluorescence microplate reader with kinetic detection, temperature control and bottom-read function
·96-well black-walled clear-bottom cell culture plates
·Complete culture medium matched to test cell line
·Centrifuge, adjustable pipettes and pipette tips

Reagent Preparation

OCR-Probe: Ready-to-use; equilibrate to room temperature (25℃) before use.

Note:Store unused probes in the dark at 4℃; DO NOT store them at -20℃, as freezing the probes will result in lower test values.

Mineral Oil: Ready-to-use; pre-heat to 37℃ before use.Pre-warm for ≥30 min.

10 mM CCCP: Dilute stock to 1 mM with complete medium, then further dilute to target concentration as needed (final well concentration: 2–5 μM). Equilibrate to room temperature (25℃) before use.

100 U/mL GOD: Ready-to-use; equilibrate to room temperature (25℃) before use

OCR-Probe Working Solution:

Dilute OCR-Probe 10–20 fold with complete medium pre-warmed to 37℃.

Note: For initial testing, use a 10-fold dilution; optimize dilution factor for individual cell lines via pilot assays. Medium must be pre-warmed to 37℃ prior to dilution.

Assay Procedure

A. Adherent Cell

1. Seed cells in 96-well black clear-bottom plates one day in advance. Set groups: Blank, GOD Control, Untreated, Drug Treatment, Positive Control (optional). Seed cells in Untreated, Drug Treatment and Positive Control wells at 5–15×10⁴ cells/well; cell viability ≥98% via Trypan Blue staining. Leave Blank wells cell-free.However, it is important to include one GOD well and two background (Blank) wells in the assay. It is suggested that the Untreated, Drug Treatment and Positive Control be assayed in triplicate.

Group Definition

(1) Blank: No cells, reflects intrinsic probe fluorescence signal; these values are excluded from final OCR calculation.

(2) GOD: Cell-free wells supplemented with GOD solution, serves as a maximum-oxygen-depletion reference control to validate probe oxygen-sensing performance.

(3) Untreated: Cell-seeded wells, for measuring basal oxygen consumption rate.

(4) Drug Treatment: Cell-seeded wells supplemented with test compound, to evaluate drug effects on cellular respiration.

(5) Positive Control (optional): Cell-seeded wells supplemented with medium-diluted CCCP, positive reference for elevated cellular oxygen consumption.

Note:

(1) The Drug Treatment group can be configured according to your specific experimental requirements. The CCCP uncoupler provided in this kit can be added directly for detection after adding the OCR-Probe working solution and incubating; however, for other drugs, the addition timing must be determined based on your specific experimental model

a. If the drug is a classic mitochondrial respiratory chain-related agent such as CCCP/FCCP, Antimycin A, Oligomycin, or Rotenone, follow the standard experimental procedure (add the drug in Step 5).

b. For classic drugs not related to the mitochondrial respiratory chain, add the drug before Step 3; for the Drug Treatment group, add 10 μL of pre-warmed complete medium in Step 5

c. For drugs that induce cell differentiation, it is recommended to induce the cells prior to seeding (before Step 1) and then perform the cellular OCR assay. For the Drug Treatment group, add 10 μL of pre-warmed complete medium during Step 5.

(2) The specific cell density should be determined based on the cell line. It is recommended to first conduct a preliminary experiment with a gradient of cell densities and select the cell density yielding the best results for the model experiment. The following are the cell density ranges tested with this kit: for reference only: Hela cells 5–15×10⁴cells/well, HEK293T cells 6–12×10⁴ cells/well, HepG2 cells 6–12×10⁴cells/well.

2. Allow all reagents to equilibrate to room temperature (25℃). Before starting the experiment, the complete culture medium and Mineral Oil must be pre-warmed at 37℃ for at least 30 minutes. Set the microplate reader to 37℃ in advance to ensure that the entire assay is conducted at 37℃.

3. Preparation of OCR-Probe Working Solution: Dilute the OCR-Probe 10–20-fold with the corresponding cell complete culture medium that has been preheated to 37℃ before use.

Note: For preliminary testing, it is recommended to first dilute the OCR-Probe 10-fold with preheated medium. For different cell lines, conduct preliminary experiments first and then adjust the dilution ratio based on actual conditions.

4. Discard the supernatant. Add 100 µL of OCR-Probe Working Solution to each well in the Blank, GOD, Untreated group, Drug Treatment, and Positive Control (optional). Place the microplate in a preheated microplate reader and incubate at 37℃ for 10 minutes.

5. Add 10 µL of pre-warmed complete medium to each well in the Blank and Untreated. Add 10 µL of CCCP diluted with complete medium (final concentration in the well: 2–5 µM) to each well in the Positive Control (optional). Add 10 µL of the drug diluted with complete medium to each well in the Drug Treatment. Add 10 µL of 100 U/mL GOD to the GOD group as a control for maximum oxygen consumption by the probe; this directly demonstrates whether the probe effectively detects oxygen consumption.

Note: If the initial fluorescence signal of GOD Control is excessively high, dilute the 100 U/mL GOD stock solution before adding to wells Glucose oxidase (GOD) consumes dissolved oxygen in the medium, thereby directly illustrating the sensitivity of the probe in detecting extracellular dissolved oxygen.

6. Add 2 drops of Mineral Oil preheated to 37℃ to each well (you can cut off a portion of the pipette tip with scissors to draw up 100 µL for dispensing). Immediately place the microplate in the fluorescence microplate reader, set the detection temperature to 37℃, the excitation wavelength to 405 nm, and the emission wavelength to 650 nm. Select the bottom-reading mode and run the assay in kinetic mode for 120 minutes, with readings taken every 2–5 minutes

Note: To ensure timely detection, it is recommended to set up the instrument's detection program in advance. After adding mineral oil, place the plate in the instrument immediately to avoid prolonged exposure of the system to air, which could cause changes in dissolved oxygen concentration. If the number of wells is high (more than 48 wells), and the instrument requires more than 2 minutes to complete a full run, the interval between readings may be extended to 5 minutes; however, it is recommended not to exceed 5 minutes.After adding Mineral Oil, the microplate must be kept at a constant temperature of 37℃ inside the instrument and must not be removed; it must remain in the instrument for incubation and testing throughout the entire assay. Shaking the instrument often leads to unstable results and is not recommended.

Reference Table for Adherent Cell Assays

	Blank	GOD	Untreated	Drug Treatment	Positive Control
Cell Seeding Density	--	--	5-15×10^4 cells/well	5-15×10^4 cells/well	5-15×10^4 cells/well
OCR-Probe Working Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Place the microplate in a preheated fluorescence microplate reader and incubate at 37℃ for 10 min					
Preheated complete medium	10 µL	--	10 µL	--	--
Drug diluted with complete medium	--	--	--	10 µL	--
CCCP diluted with complete medium (final concentration in well: 2–5 µM)	--	--	--	--	10 µL
100 U/mL GOD	--	10 µL	--	--	--
Mineral Oil	2 drops/100 µL	2 drops/100 µL	2 drops/100 µL	2 drops/100 µL	2 drops/100 µL

Immediately after adding Mineral Oil to the well, place the microplate in a fluorescence microplate reader. Set the detection temperature to 37℃, the excitation wavelength to 405 nm, and the emission wavelength to 650 nm. Select the bottom-reading mode and run the kinetic assay for 120 minutes, with readings taken every 2–5 minutes. To ensure timely detection, it is recommended to set up the instrument's detection program in advance. Please begin detection immediately after adding Mineral Oil to avoid prolonged exposure of the system to air, which can cause changes in dissolved oxygen concentration. If the number of wells is high (more than 48 wells), some instruments may require more than 2 minutes to complete a full run; in such cases, the interval between readings may be extended to 5 minutes, though it is recommended not to exceed 5 minutes.

B. Suspended Cell

1. Allow all reagents to equilibrate to room temperature (25℃). Before starting the assay, the complete culture medium and Mineral Oil must be pre-warmed at 37℃ for at least 30 minutes. Set the microplate reader to 37℃ in advance to ensure that the entire assay is conducted at 37℃.

2. Preparation of OCR-Probe Working Solution: Dilute the OCR-Probe 10–20-fold with the corresponding cell complete medium that has been preheated to 37℃ before use.

Note: For preliminary testing, it is recommended to first dilute the OCR-Probe 10-fold with pre-warmed medium. For different cell lines, conduct preliminary experiments first and then adjust the dilution ratio based on actual conditions.

3. Centrifuge the cells to collect them, discard the supernatant, and resuspend the cells in the OCR-Probe Working Solution. The cell concentration in each tube should be approximately 5–10×10^6 cells/mL.

Note: The specific cell density may vary depending on the cell line and should be determined experimentally. It is

recommended to first conduct a preliminary experiment with a gradient of cell densities and select the cell density yielding the best results for the main experiment. The following are the cell density ranges tested with this kit, provided for reference only: Jurkat cells 5–10×10⁶ cells/mL; the cell concentration when 100 µL is added to a well is 5–10×10⁵ cells per well.

4. Set up a black-bottomed microplate with Blank, GOD , Untreated, Drug Treatment, Positive Control (optional) wells. Add 100 µL of OCR-Probe Working Solution to the Blank and GOD wells. Add 100 µL of cell suspension resuspended in OCR-Probe Working Solution (approximately 5–10 × 10⁵ cells per well) to each well in the Untreated, Drug Treatment, and Positive Control group (optional). Place the microplate in a fluorescence microplate reader and incubate at 37℃ in the dark for 10 minutes. It is recommended to set up three replicates for the Untreated group, Drug Treatment group, and Positive Control group; two replicates for the Blank group; and one well for the GOD group

Group Definition

(1) Blank: No cells, reflects intrinsic probe fluorescence signal; these values are excluded from final OCR calculation.

(2) GOD: Cell-free wells supplemented with GOD solution, serves as a maximum-oxygen-depletion reference control to validate probe oxygen-sensing performance.

(3) Untreated: Cell-seeded wells, for measuring basal oxygen consumption rate.

(4) Drug Treatment: Cell-seeded wells supplemented with test compound, to evaluate drug effects on cellular respiration.

(5) Positive Control (optional): Cell-seeded wells supplemented with medium-diluted CCCP, positive reference for elevated cellular oxygen consumption.

Note:

The Drug Treatment group can be configured according to your specific experimental requirements. The CCCP uncoupler provided in this kit can be added directly for detection after adding the OCR-Probe working solution and incubating; however, for other drugs, the addition timing must be determined based on your specific experimental model

a. If the drug is a classic mitochondrial respiratory chain-related agent such as CCCP/FCCP, Antimycin A, Oligomycin, or Rotenone, follow the standard experimental procedure (add the drug in Step 5).

b. For classic drugs not related to the mitochondrial respiratory chain, add the drug before Step 3; for the Drug Treatment group, add 10 µL of pre-warmed complete medium in Step 5

c. For drugs that induce cell differentiation, it is recommended to induce the cells prior to seeding (before Step 1) and then perform the cellular OCR assay. For the Drug Treatment group, add 10 µL of pre-warmed complete medium during Step 5.

5. Add 10 µL of pre-warmed complete medium to each well in the Blank and Untreated ; add 10 µL of CCCP diluted with complete medium (final concentration in the well: 2–5 µM) to each well in the Positive Control (optional); and add 10 µL of the drug diluted with complete medium to each well in the Drug Treatment. Add 10 µL of 100 U/mL GOD to the GOD group as a control for maximum oxygen consumption by the probe; this directly demonstrates whether the probe effectively detects oxygen consumption.

Note: If the initial value in the GOD group is too high, dilute the solution before adding it to the well. Glucose oxidase (GOD) consumes dissolved oxygen in the medium, thereby directly indicating the sensitivity of the probe in detecting extracellular dissolved oxygen.

6. Add 2 drops of Mineral Oil preheated to 37℃ to each well (you can cut off a portion of the pipette tip with scissors to draw up 100 µL for dispensing). Immediately place the microplate in the fluorescence microplate reader, set the detection temperature to 37℃, the excitation wavelength to 405 nm, and the emission wavelength to 650 nm. Select the bottom-reading mode and run the assay in kinetic mode for 120 minutes, with readings taken every 2–5 minutes**.**

Note: To ensure timely detection, it is recommended to set up the instrument's detection program in advance. After adding mineral oil, place the plate in the instrument immediately to avoid prolonged exposure of the system to air, which could cause changes in dissolved oxygen concentration. If the number of wells is high (more than 48 wells), and the instrument requires more than 2 minutes to complete a full run, the interval between readings may be extended to 5 minutes; however, it is recommended not to exceed 5 minutes.After adding Mineral Oil, the microplate must be kept at a constant temperature of 37℃ inside the instrument and must not be removed; it must remain in the instrument for incubation and testing throughout the entire assay. Shaking the instrument often leads to unstable results and is not recommended.

Reference Table for Suspended Cell Assays

	Blank	GOD	Untreated	Drug Treatment	Positive Control
Cell Seeding Density	--	--	5-10×10^5 cells/well	5-10×10^5 cells/well	5-10×10^5 cells/well
OCR-Probe Working Solution	100 µL	100 µL	--	--	--
Cell suspension resuspended using OCR-Probe Working Solution	--	--	100 µL	100 µL	100 µL
Place the microplate in a preheated fluorescence microplate reader and incubate at 37℃ for 10 min					
Preheated complete medium	10 µL	--	10 µL	--	--
Drug diluted with complete medium	--	--	--	10 µL	--
CCCP diluted with complete medium (final concentration in well: 2–5 µM)	--	--	--	--	10 µL
100 U/mL GOD	--	10 µL	--	--	--
Mineral Oil	2 drops/100 µL	2 drops/100 µL	2 drops/100 µL	2 drops/100 µL	2 drops/100 µL

Immediately after adding Mineral Oil to the well, place the microplate in a fluorescence microplate reader. Set the detection temperature to 37℃, the excitation wavelength to 405 nm, and the emission wavelength to 650 nm. Select the bottom-reading mode and run the kinetic assay for 120 minutes, with readings taken every 2–5 minutes. To ensure timely detection, it is recommended to set up the instrument's detection program in advance. Please begin detection immediately after adding Mineral Oil to avoid prolonged exposure of the system to air, which can cause changes in dissolved oxygen concentration. If the number of wells is high (more than 48 wells), some instruments may require more than 2 minutes to complete a full run; in such cases, the interval between readings may be extended to 5 minutes, though it is recommended not to exceed 5 minutes.

C. Calculation of OCR Results

1. Calculate the average of the fluorescence readings for each replicate well in each group, and plot a curve with fluorescence values on the y-axis and time (min) on the x-axis.

2. Identify and select the interval on the curve that exhibits the best linearity, and perform a linear regression fit. The slope of the regression equation is the OCR, expressed in RFU/min (Relative Fluorescence Units per minute).

Sample Results

1. One day in advance, seed HeLa cells into a 96-well black-bottomed cell culture plate, setting up a Blank group, a GOD group, an Untreated group, a Positive Control group, and a Drug Treatment group. Seed 5–15×10⁴ cells/well Hela cells into each well of the Untreated group, Positive Control group, and Drug Treatment group; after counting with trypan blue, the cell viability should be 98% or higher. No cells were seeded in the Blank group. The Untreated group, Positive Control group, and Drug Treatment group each had three replicate wells; the Blank group had two replicate wells; and the GOD group had one well.
2. All reagents should be equilibrated to room temperature (25℃). Prior to the experiment, the complete medium and Mineral Oil must be pre-warmed at 37℃ for 60 minutes. The fluorescence microplate reader should be preset to 37℃ to ensure that the entire assay is conducted at 37℃.
3. Preparation of OCR-Probe Working Solution: Dilute the OCR-Probe 10-fold with DMEM complete medium that has been pre-warmed to 37℃.
4. Discard the supernatant. Add 100 µL of Probe Working Solution to each well in the blank group, GOD group, Untreated group, Positive Control group (CCCP), and Drug Treatment group (Antimycin A). Place the microplate in the fluorescence microplate reader and incubate at 37℃ in the dark for 10 minutes.
5. Add 10 µL of pre-warmed complete medium to each well in the Blank and Untreated groups; add 10 µL of CCCP diluted with complete medium (final concentration in the well: 2 µM) to each well in the Positive Control group; and add 10 µL of Antimycin A diluted with complete medium (final concentration in the well: 1 µM) to each well in the Drug Treatment group. For the GOD group, add 10 µL of 100 U/mL GOD as a control for the OCR-Probe's maximum oxygen consumption, which directly indicates whether the probe effectively detects oxygen consumption.
6. Calculate the average of the fluorescence readings for duplicate wells in each group, and plot a curve with fluorescence values on the y-axis and time (min) on the x-axis, as shown in Figure 1.
7. Identify and select the linear segment of the curve, then perform a linear regression to calculate the slope, as shown in Figure 2. The slope obtained from the regression is the OCR, expressed in RFU/min (Relative Fluorescence Units per minute), as shown in Figure 3.

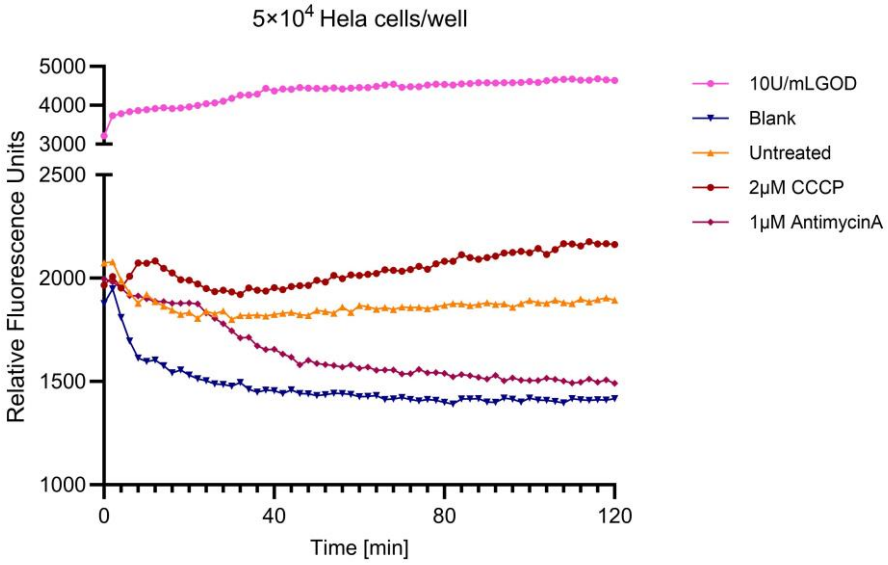

Figure 1. Kinetic Curve of Extracellular Oxygen Consumption in Hela Cells.

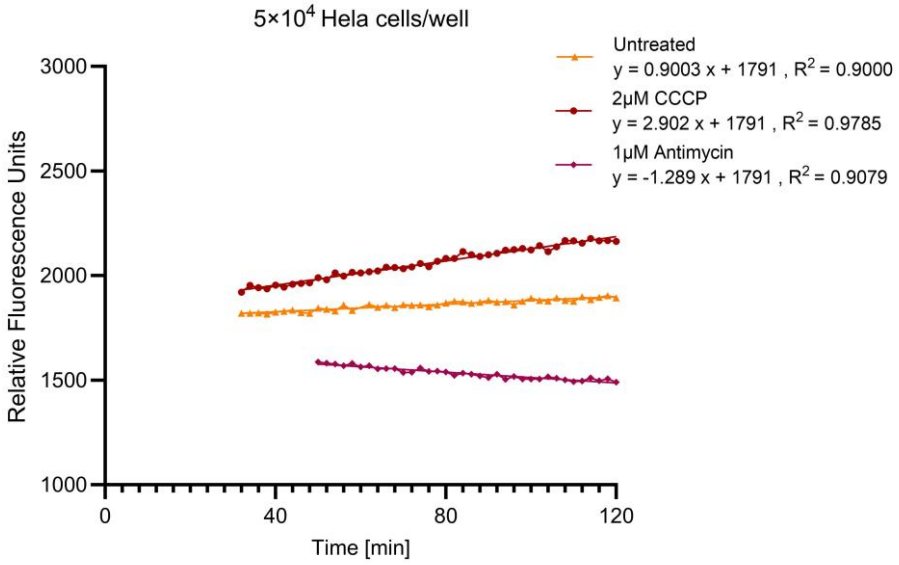

Figure 2. Linear regression fit of fluorescence values over time in the kinetic curve of extracellular oxygen consumption in Hela cells

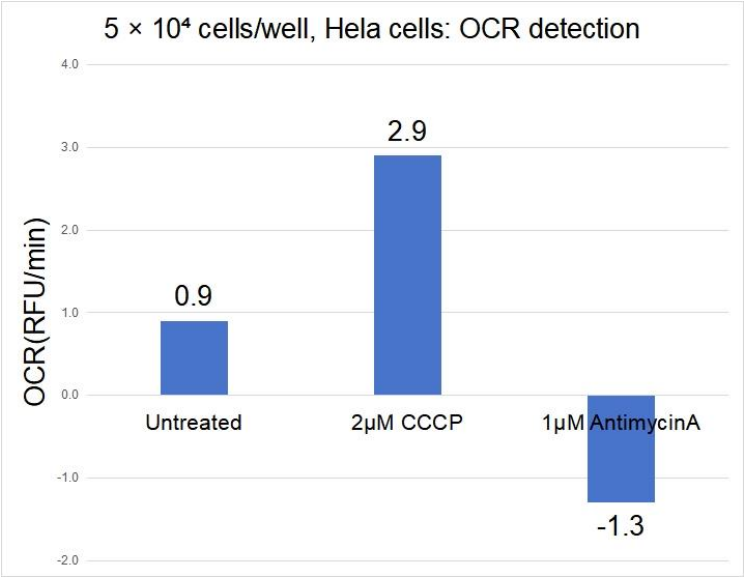

Figure 3. Comparison of OCR Values Among Different Groups of Hela Cells

FAQ

1. Q: No trend observed in the untreated group?

A: This may be due to the following reasons:

① Insufficient cell number or poor cell condition.

Cells must be counted using trypan blue prior to the experiment, and the cell viability must be above 98%. Cells seeded in a 96-well plate should appear fused when observed under a microscope. If gaps are observed between cells, this indicates an insufficient cell number, and the seeding density must be increased.

② The temperature was not maintained at a constant 37℃ during the assay.

After adding mineral oil, the microplate must be placed in the instrument and kept at a constant 37℃; it must not be removed. The microplate must remain in the instrument for incubation and detection throughout the entire assay. Shaking the instrument often leads to unstable results and is not recommended.

③ Check whether the reagents were pre-warmed to 37℃ in advance. Before the experiment, the complete medium and mineral oil should be pre-warmed to 37℃ for at least 30 minutes, and the microplate reader should be set to 37℃ prior to testing. It is recommended to incubate the microplate in the microplate reader; incubation in a 37℃ incubator is not recommended.

2. Q: Can I use serum-free basal medium?

A: We recommend using complete medium containing serum for your experiments, as fluorescence readings tend to be lower in serum-free medium compared to serum-containing medium. If you wish to compare results, be sure to use the same medium conditions in every experiment.

3. Q: How do drugs such as CCCP in the assay kit affect the cellular oxygen consumption rate (OCR)?

A: Common drugs used to interfere with cellular oxidative phosphorylation include CCCP/FCCP, Antimycin A, and others. CCCP and FCCP are classic oxidative phosphorylation uncouplers that dissipate the transmembrane proton gradient across the inner mitochondrial membrane, thereby uncoupling electron transport from ATP synthesis; Following drug treatment, mitochondrial electron transport is not blocked, and the oxygen consumption rate increases; therefore, the cellular OCR rises significantly. Antimycin A is an inhibitor of mitochondrial respiratory chain complex III; it blocks the mitochondrial electron transport chain and directly inhibits mitochondrial respiratory function, resulting in a marked decrease in cellular OCR after administration.

4. Q: In OCR assays, can multiple drugs be added to the same well for analysis?

A: No. The assay system of this kit requires that only one test drug be added per well; it does not support the mixing of multiple drugs in the same well to detect cellular oxygen consumption. If you need to evaluate the effects of multiple drugs on cellular OCR, you must set up separate drug-treatment wells and conduct parallel assays.

Recommended Products

Catalog No.	Product Name
KTA9011	Extracellular Acidification Rate (ECAR) Assay Kit
KTA9012	Mitochondrial Superoxide Assay Kit with MitoNeoD
KTA4001	Mitochondrial Membrane Potential Assay Kit (JC-1)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.