Oxygen Consumption Rate (OCR) Assay Kit

Cat. No. KITG-092 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The Oxygen Consumption Rate (OCR) Assay Kit is a fluorescent assay kit designed to detect changes in extracellular oxygen consumption in cells using a specific oxygen-sensitive fluorescent probe. It enables convenient and direct measurement of oxygen consumption through simple mixing and detection with a fluorescence microplate reader. Oxygen consumption analysis is a useful tool for studying metabolism. Oxygen consumption is a key indicator of cellular metabolic function, particularly mitochondrial function. Investigating live-cell metabolism through this indicator can provide in-depth insight into cellular function and the role of metabolic changes in disease progression.
Description	Mitochondrial function is critical for cellular metabolism, survival, and energy production. Impaired mitochondrial respiration is associated with pathological states such as cancer, ischemia-reperfusion injury, and neurodegenerative diseases. Mitochondrial toxicity is also a common cause of safety-related drug resistance and withdrawal symptoms. The kit can be used for direct, real-time analysis of cellular respiration and mitochondrial function. It is applicable to whole cells, including adherent and suspension cells; permeabilized cells; 3D cultures; tissues; small organisms; spheroids; scaffolds; matrices; isolated mitochondria; isolated enzymes; bacteria; yeast; and molds for extracellular oxygen-consumption measurement. Assay Principle The kit uses a specific oxygen-sensitive fluorescent probe to detect changes in extracellular oxygen consumption. It enables real-time oxygen-consumption analysis of whole cells or isolated mitochondria and provides a reliable, high-throughput approach for assessing cellular respiration, metabolic characteristics, and treatment-related mitochondrial toxicity. During respiration, dissolved oxygen is

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consumed and the oxygen concentration in the sample decreases, resulting in an increased fluorescence signal. The oxygen-sensitive fluorescent probe has a maximum excitation wavelength of 455 nm and a maximum emission wavelength of 603 nm. Its fluorescence is quenched by O_2 through molecular collisions with oxygen. Fluorescence intensity is higher under anaerobic conditions and lower under aerobic conditions. Therefore, fluorescence intensity is inversely proportional to the amount of extracellular O_2 in the sample. The oxygen consumption rate is determined from the change in fluorescence signal over time. Increased cellular oxygen consumption produces a greater rate of fluorescence-signal increase, whereas decreased oxygen consumption produces a lower rate of signal change. The probe reaction with O_2 is non-destructive and completely reversible. The probe has no cytotoxicity, is chemically stable, inert, water-soluble, and cell-impermeable, and cannot penetrate intact cell membranes. It may be used simultaneously with glycolysis assays, mitochondrial membrane-potential JC-1 assays, ROS assays, cellular ATP assays, LDH assays, and other detection reagents for multi-parameter analysis. The probe uses visible-light excitation; compared with UV-excited detection reagents, it causes less cell damage and lower cytotoxicity, providing more accurate and objective results. Form: Liquid. Size: 200 T / 400 T. Based on a 96-well cell-culture plate, the 200 T package can detect 200 samples. Detection Method: Fluorescence microplate reader. Excitation wavelength: 455-468 nm. Emission wavelength: 603 nm. The probe maximum is 455 nm excitation and 603 nm emission. The optimal excitation range is 450-470 nm, and the emission range is 586-610 nm or 590-610 nm. Compatible Sample Types: Adherent cells, suspension cells, permeabilized cells, isolated mitochondria, 3D cultures, tissues, isolated enzymes, bacteria, yeast, molds, small organisms, spheroids, scaffolds, and matrices. Features and Benefits 1. Widely applicable to a range of in vitro models, including monolayer cells, suspension cells, microorganisms, and

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	specific 3D cultures. 2. The simple mix-and-detect protocol supports multi-parameter analysis with other assay systems. 3. The reversible probe allows observation of transient and long-term changes in oxygen consumption. 4. Compatible with most fluorescence microplate readers and standard 96-well and 384-well plates. 5. Supports convenient high-throughput measurement without dedicated oxygen-consumption equipment or a large capital investment. 6. Visible-light excitation reduces cell damage and cytotoxicity compared with UV-excitation methods. 7. The probe is non-cytotoxic, chemically stable, inert, water-soluble, and cell-impermeable. 8. The method requires only a fluorescence plate-reading device such as a fluorescence microplate reader.
Form	Liquid
Applications	Mitochondrial function and cellular metabolism research. Measurement of mitochondrial activity in live cells. Study of the effects of treatments or genetic modification on cellular oxygen consumption. Study of respiration and mitochondrial function. Investigation of the relationship between oxygen content and changes in cellular metabolism. Direct assessment of the mitochondrial toxicity of drug treatments. Applicable samples include adherent cells, suspension cells, permeabilized cells, isolated mitochondria, 3D cultures, tissues, isolated enzymes, bacteria, yeast, and molds.
Notes	General Notes 1. After opening, store each component under the required conditions and use the reagents as soon as possible. 2. Prevent oxidation. 3. Before formal experiments, select several samples for preliminary experiments to optimize the experimental conditions and obtain the best results. 4. For reagents supplied in screw-cap microcentrifuge tubes, briefly centrifuge the tubes before opening to collect liquid from the cap and tube wall at the bottom and avoid reagent loss. 5. Do not mix the kit reagents with reagents from other sources because this may affect assay

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performance. 6. Bacterial or fungal contamination of samples or reagents, or cross-contamination among reagents, may produce erroneous results. 7. Disposable pipette tips, tubes, bottles, or glassware are recommended. Reusable glassware must be cleaned thoroughly and be free of residual detergent before use. 8. After the experiment, process all samples and contacted utensils according to the prescribed procedures. 9. Temperature has a significant effect on detection. Use a microplate reader equipped with temperature control whenever possible. 10. Cell handling must be performed carefully to minimize artificial cell damage. 11. The described procedure uses a 96-well cell-culture plate as an example. For other culture containers or devices, adjust reagent volumes and the detection method according to the actual conditions, maintaining proportional reagent volumes. 12. The signal change of the oxygen fluorescent probe directly depends on the respiratory rate of the tested cell type. Use a high cell density per well as a starting point and reduce the cell number as needed. Not all cell types consume enough oxygen for detection. 13. Use fluorescence-specific transparent-bottom black culture plates to reduce optical interference between wells. 14. Preheat all instruments, reagents, solutions, and consumables used for measurement to 37°C. 15. Avoid generating bubbles when adding the oxygen sealing solution. Use a multichannel pipette whenever possible. 16. If the test compound has red fluorescence, such as doxorubicin, replace the medium and perform detection in HBSS or PBS under serum-free and phenol-red-free conditions. 17. FCCP-treated positive-control samples are recommended. 18. For conventional fluorescence-intensity measurement, use a monochromator-based or filter-based microplate reader. The optimal excitation wavelength is 450-470 nm and the emission wavelength is 586-610 nm. Select the wavelength producing the strongest signal according to the actual instrument and samples. 19. Detection gain parameters, including PMT parameters, are generally set to medium or high. 20. Either bottom reading or top reading may be used. Evaluate both modes in preliminary experiments for the specific cell model and use the mode showing the clearest trend. 21. The oxygen sealing solution may be added with a pipette along the wall of the culture plate or directly from a dropper bottle at two drops per well. When

using a pipette, the yellow pipette tip may be cut at a 45° angle, 3-4 mm from the tip. A slight variation in sealing-solution volume between 90 and 110 µL does not adversely affect detection. 22. Some cell models may initially show a short decrease in fluorescence intensity, usually for approximately 10 minutes. Use the time point at which fluorescence begins to increase after equilibration as the starting point. 23. Fluorescence intensity values must be blank-corrected. Select the linear part of each signal curve, excluding the initial lag phase and the late plateau phase. Use suitable analysis software or spreadsheet-based linear regression to determine the slope, representing OCR, for each well, and calculate the mean among replicate wells. Cell Seeding and Plate Setup 1. For adherent cells, seed cells in a 96-well plate with 200 µL medium and incubate overnight in a 37°C CO_2 incubator. 2. For suspension cells, seed cells in 100 µL medium on the day of detection. 3. The required seeding density depends on the cell type. Perform a cell-density preliminary experiment to determine the optimal density. Beginning from a high cell density, generally 60,000 cells, the typical optimal numbers per well in a 96-well plate are approximately 80,000 cells for adherent cells and 5-6 × 10^5 cells for suspension cells. 4. Set three replicate wells for every test sample. 5. Leave at least six wells on each 96-well plate without cells for blank-control and signal-control wells. Kit Components Component A: Oxygen Consumption Fluorescent Probe. 200 T: 1 mL. 400 T: 1 mL × 2. Storage: Store at -20°C, sealed and protected from light. Avoid repeated freeze-thaw cycles. Component B: Oxygen Sealing Solution. 200 T: 20 mL. 400 T: 50 mL. Storage: Store at room temperature. Instruction manual: 1 copy. Materials Required but Not Supplied Instruments: Fluorescence microplate reader equipped with the corresponding wavelength filters and a temperature controller; centrifuge; pipette; refrigerator; and ice box. Reagents: PBS buffer, pH 7.4, commonly used as 10 mM phosphate-buffered saline, 1×, containing approximately 8 mM Na_2HPO_4, 2 mM KH_2PO_4, 137 mM NaCl, and 3 mM KCl; or HBSS containing calcium, magnesium, and glucose and without phenol red. The specified HBSS composition is $CaCl_2$, anhydrous, 140 mg/L (1.261 mM);

	$MgCl_2·6H_2O$, 100 mg/L (0.493 mM); $MgSO_4·7H_2O$, 100 mg/L (0.407 mM); KCl, 400 mg/L (5.333 mM); KH_2PO_4, 60 mg/L (0.441 mM); $NaHCO_3$, 350 mg/L (4.167 mM); NaCl, 8000 mg/L (137.931 mM); Na_2HPO_4, anhydrous, 48 mg/L (0.338 mM); and D-glucose, 1000 mg/L (5.556 mM). Control reagents: FCCP, glucose oxidase, and antimycin A. Consumables: Black transparent-bottom fluorescence-specific 96-well plate; centrifuge tubes; pipette tips; and disposable gloves. Safety Warning For Research Use Only. Not for use in diagnostic, food, or cosmetic testing procedures. Wear an appropriate laboratory coat and disposable gloves. Avoid contact of reagents with the skin or mucous membranes. If a reagent contacts the skin or eyes, rinse immediately with water.
Storage	Stability: The unopened kit is stable for 6 months when stored under the required conditions. Refer to the product packaging label for the specific expiration date. Use the reagents as soon as possible after opening. Component A, Oxygen Consumption Fluorescent Probe: Store at -20°C, sealed and protected from light. Avoid repeated freeze-thaw cycles. Component B, Oxygen Sealing Solution: Store at room temperature. Complete kit: Store at 2-8°C, protected from light.
Shipping	Ship on ice. After receipt, store Component B at 4°C. Store Component A at -20°C, sealed and protected from light, and avoid repeated freeze-thaw cycles.
Reaction conditions	Preparation 1. Prepare the detection working solution by diluting the oxygen fluorescent probe 25-fold with the corresponding medium. For example, mix 96 µL fresh medium with 4 µL oxygen fluorescent probe. Mix thoroughly. The optimal final dilution of the O_2 fluorescent probe is 25-fold. 2. Set up the wells as follows. Blank wells: Include cells and add 100 µL fresh medium plus 10 µL solvent. Do not add oxygen fluorescent probe. Control wells: Include cells and add 100 µL detection working solution plus 10 µL solvent. Sample wells: Include cells and add 100 µL detection working solution plus 10 µL test compound. All wells: Add 100 µL oxygen

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sealing solution. Optional cell-free negative control: No cells; add 100 µL detection working solution. Optional cell-free positive control: Add 86 µL fresh medium, 10 µL of 1 mg/mL glucose oxidase aqueous solution, and 4 µL oxygen fluorescent probe. Optional cell negative control: Include cells; add 95 µL fresh medium, 1 µL of 100 µM antimycin A stock solution, and 4 µL oxygen fluorescent probe. Optional cell positive control: Include cells; add 95 µL fresh medium, 1 µL of 0.1-5 mM FCCP stock solution, and 4 µL oxygen fluorescent probe. If FCCP is used, perform a dose test because cells exhibit a bell-shaped response to FCCP. 3. Prepare the cell models according to the experimental requirements. Include blank wells, control wells, and replicate wells. 4. Remove the culture medium from the cultured cells to be tested and wash the cells with PBS. 5. Add 100 µL freshly prepared detection working solution. 6. Incubate the plate in a microplate reader at 37°C or in an incubator for 30 minutes. Assay Protocol The following procedure uses adherent cells as an example. For suspension cells, add the corresponding cell suspension and follow the same subsequent steps. Perform all liquid-exchange and cell-washing steps carefully to avoid cell detachment and loss. Collect detached and floating cells by centrifugation and return them to the corresponding wells. 1. Prepare the detection working solution by diluting the oxygen fluorescent probe 25-fold with the corresponding medium, for example, 96 µL fresh medium plus 4 µL oxygen fluorescent probe. Mix thoroughly. 2. Set up blank, control, and sample wells. Blank wells contain cells, 100 µL fresh medium, and 10 µL solvent without oxygen fluorescent probe. Control wells contain cells, 100 µL detection working solution, and 10 µL solvent. Sample wells contain cells, 100 µL detection working solution, and 10 µL test compound. Add 100 µL oxygen sealing solution to all wells. Keep the amount of added test compound low to minimize potential solvent effects. The actual addition volume may be adjusted, but it must be consistent among wells. 3. Prepare the cell models and corresponding experimental design according to the study requirements. Include blank wells, control wells, and replicate wells. The optional controls described above are recommended when applicable. 4. Remove the culture medium from the cultured cells and wash the

cells with PBS. 5. Add 100 µL freshly prepared detection working solution. 6. Incubate the plate in a microplate reader at 37°C or in an incubator for 30 minutes. 7. If required, add the test compounds at this stage, generally 1-10 µL according to the actual conditions. Keep the added compound volume low to minimize potential solvent effects. 8. Immediately after adding the test compounds, add 100 µL oxygen sealing solution to each well. Accurate pipetting is recommended, or add two drops directly with a dropper bottle. Add the sealing solution slowly along the wall of the culture plate without rapidly inserting the tip below the medium surface. Avoid bubbles. A volume variation of 90-110 µL does not adversely affect detection. 9. Detect changes in extracellular O_2 consumption. Place the plate in a microplate reader at 37°C and begin detection after 5 minutes of equilibration. Set excitation to 455-468 nm and emission to 603 nm. Measure fluorescence intensity every 3, 5, or 10 minutes. The usual detection period is 120 or 180 minutes. Fluorescence intensity increases with extracellular O_2 consumption. Use fluorescence-specific transparent-bottom black culture plates. Some cell models may show a short initial decrease in fluorescence, generally for approximately 10 minutes; use the point at which fluorescence begins to increase after equilibration as the starting point. The optimal excitation range is 450-470 nm and the emission range is 590-610 nm; select the wavelength producing the strongest signal for the instrument and samples. 10. Plot the oxygen-consumption curve with fluorescence value, F, on the vertical axis and time in minutes on the horizontal axis. Select the linear portion and compare cellular OCR according to the slope of that portion. Use blank-corrected fluorescence values. 11. If required, calculate the slope for each sample. Select the appropriate linear portion of the signal curve, excluding the initial lag phase and late plateau. Use suitable analysis software or spreadsheet-based linear regression to determine the OCR slope for each well, and calculate the average among replicate wells. Assay Time Incubation: 30 minutes. Detection: generally

	120 or 180 minutes. Reading interval: every 3, 5, or 10 minutes.
Unit definition	Analysis Plot the oxygen-consumption-rate curve using fluorescence value, F, as the vertical coordinate and time, minutes, as the horizontal coordinate. Select the linear portion of the curve and compare cellular OCR using the slope of that portion. Fluorescence intensity values must be blank-corrected. Simple calculation formula: $OCR = [(F_sample_T_2 - F_sample_T_1) - (F_blank_T_2 - F_blank_T_1)] \div (T_2 - T_1)$ Where: $F_sample_T_2$: Fluorescence intensity of the sample well at time point 2. $F_sample_T_1$: Fluorescence intensity of the sample well at time point 1. $F_blank_T_2$: Fluorescence intensity of the blank well at time point 2. $F_blank_T_1$: Fluorescence intensity of the blank well at time point 1. T_2: Time point 2, minutes. T_1: Time point 1, minutes. For dose-response curve generation, plot the calculated OCR against the corresponding compound concentration to generate the dose-response curve.
Usage	For Research Use Only. Not for use in diagnostic, food, or cosmetic testing procedures.

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