CREATIVE BIOMART®
Assay Kit

Oxygen Consumption Rate Assay Kit

Product Information

Common Name

OCR

Cat.No. Kit-1991

Description

The oxygen consumption rate (OCR) of cells is an important indicator of normal cellular function. It is used as a parameter to study mitochondrial function as well as a marker of factors triggering the switch from healthy oxidative phosphorylation to aerobic glycolysis in cancer cells. Oxygen consumption is traditionally measured by a cumbersome oxygen electrode, a specialized piece of equipment that typically yields low sample throughput. The phosphorescent oxygen probe offers a novel method for analyzing oxygen consumption in whole cells. The cell-based Oxygen Consumption Rate Assay Kit utilizes this newly developed phosphorescent oxygen probe to measure oxygen consumption rate in living cells. Antimycin A, an inhibitor of the mitochondrial electron transport chain, is included to be used as a positive control. Glucose oxidase is also included in the kit to be used as a reference for oxygen depletion. The kit is easy to use and can be easily adapted to high throughput screening for compounds which modulate oxygen consumption rate.

Stability

1 year

Storage

-20°C

Synonyms

Measure OCR without the need for an oxygen electrode; Utilize MitoXpress -Xtra, a phosphorescent oxygen probe; Includes antimycin A, an inhibitor of oxygen consumption, as a control; Includes glucose oxidase as a reference for oxygen depletion; OCR

Size

96 wells

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Kit Components

Phosphorescent Oxygen Probe: 1 vial; -20°C
HS Mineral Oil Assay Reagent: 1 vial/15 ml; Room Temperature in the dark
Cell-Based Assay Glucose Oxidase: 1 vial/2 mg; -20°C
Cell-Based Assay Antimycin A: 1 vial/200 µl; -20°C

Materials Required but Not Supplied

1. A plate reader capable of measuring fluorescence using excitation and emission wavelengths of 380 and 650 nm, respectively, and having plate temperature control.
2. Adjustable pipettes and a repeating pipette.
3. 96-well (black) clear bottom tissue culture plates or standard clear polystyrene plates for culturing cells

Preparation

Reagent Preparation
1. Phosphorescent Oxygen Probe Solution
Prior to use, reconstitute the contents of the Phosphorescent Oxygen Probe Solution vial with 1 ml of distilled water or assay medium. The reconstituted Phosphorescent Oxygen Probe Solution solution is stable for one day when stored at 4°C. For long term storage, aliquot the reconstituted solution and store at -20°C. The Phosphorescent Oxygen Probe Solution will be stable for one month when stored at -20°C.
2. Glucose Oxidase Stock Solution
Prior to use, reconstitute the contents of the Cell-Based Assay Glucose Oxidase vial with 0.2 ml of distilled water. For long term storage, aliquot the reconstituted solution and store at -20°C. The reconstituted stock solution will be stable for two months when stored at -20°C.
3. Antimycin A Stock Solution
Prior to use, thaw the Cell-Based Assay Antimycin A vial and warm to room temperature. The Antimycin A will be stable for at least one year if stored at -20°C. NOTE: Please ensure that proper personal protective equipment is worn when handling Antimycin A.

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Assay Protocol

Typical Instrument Set Up

NOTE: Instrument settings will vary between manufacturers.

1. Set the plate reader temperature control to 37°C.
2. Optimal wavelengths are 380 ±20 nm for excitation and 650 ±20 nm for emission.
3. For TR-F or ratiometric TR-F, delay and mesurment times refer to Table 1 for the parameters best suited for your plate reader.
4. Gain should be optimized so that the fluorescent signal of MitoXpress in 21% O2 (air saturated) buffer is equal to 20% of the maximum detectable signal.

Instrument Signal Optimization

To optimize the signal, the following steps should be performed. For standard measurements or TR-F measurements, a signal to blank ratio >3 is required. For ratiometric TR-F (lifetime) measurement, a signal to blank ratio >10 is required for W2.

1. In a spare black, clear bottom 96-well tissue culture treated plate, add 140 µl of culture medium to six wells.
2. Add 10 µl of culture medium to three wells. These are your blank signal wells.
3. Add 10 µl of Phosphorescent Oxygen Probe Solution to three wells. These are your signal wells.
4. Gently overlay each well with 100 µl of HS Mineral Oil. The use of a repeating pipette is preferred.
5. Read the plate immediately. The plate should be measured kinetically for 30 minutes to ensure the fluorescent signal is stable.
6. If required, adjust the instrument parameters to increase measurement sensitivity in order to achieve maximal S/B ratio. The following options may be helpful:

-Increase gain (or PMT) settings or flash energy
-Adjust TR-F focal height
-Repeat without phenol red or serum
-Repeat as a top, or bottom read (plate reader dependent)
-Increase volume of Phosphorescent Oxygen Probe Solution from 10 µl to 15 µl
-Contact Instrument supplier for further options

Plate Set Up

There is no specific pattern for using the wells on the plate, but it is important to include the following

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control wells containing no cells:
Blk - Background Wells containing culture medium overlaid with oil.
MX - MitoXpress Signal Wells containing culture medium plus Phosphorescent Oxygen Probe Solution overlaid with oil.
GO - Glucose Oxidase Wells containing culture medium, Glucose Oxidase Solution, and Phosphorescent Oxygen Probe Solution overlaid with oil.
Antimycin A (AA) wells contain cells treated with Antimycin A Stock Solution. Sample wells contain cells treated with experimental compounds or vehicle.
Performing the Assay
Pipetting Hints
• It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
• Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times).
• Do not expose the pipette tip to the reagent(s) already in the well.
1. Seed cells in a black, clear bottom 96-well tissue culture treated plate at a density of 40,000-80,000 cells/well in 200 µl of culture medium. NOTE: Optimal seeding density will vary based on aerobic capacity of the cell line. We recommend trying a range of cell densities to optimize oxygen consumption rates. Incubate the cells overnight using appropriate culture conditions for the experimental cell type. It is important to have nine wells with no cells for the controls described in Plate Set Up.
2. Remove spent culture medium from all wells and replace with 150 µl of fresh medium.
3. Add test compounds or the appropriate vehicle in 10 µl to Sample Wells. NOTE: To assess the effect of a compound on mitochondrial function, cells are typically treated immediately prior to measurement. Prolonged incubations with test compounds can be performed if required. After prolonged treatment remove spent culture medium from all wells and replace with 150 µl of fresh medium.
4. Add 20 µl of culture medium to the three Blk Wells.
5. Add 10 µl of Glucose Oxidase Stock Solution to the GO Wells.
6. Add 10 µl of Antimycin A Stock Solution to the AA Wells.

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7. Add 10 µl of culture medium to the MX Wells.
8. Add 10 µl of Phosphorescent Oxygen Probe Solution to every well except the three Blk Wells.
9. Gently overlay every well with 100 µl of HS Mineral Oil. The use of a repeating pipette is preferred.
NOTE: Ensure the HS Mineral Oil is pre-warmed to the measurement temperature.
10. Read the plate immediately . The plate should be measured kinetically for >120 minutes.

Wells	Culture Medium (µl)	Glucose (µl)	Oxidase (µl)	Antimycin A (µl)	Test Compounds (µl)	Extra Culture Medium (µl)	Phosphorescent Oxygen Probe (µl)
Sample	150	-	-	10		10	
Blk	150	-	-	20	-		
MX	150	-	-	10		10	
GO	150	10	-	-		10	
AA	150	-	10	-		10	

Table 2. Pipetting summary

Analysis

Plot the Phosphorescent Oxygen Probe Signal, Intensity, or Lifetime versus Time (mins). Select the linear portion of the signal profiles and apply linear regression to determine the slope for each of the signal profiles. (This approach is preferable to calculating a slope from averaged profiles.)
Tabulate the slope values for each sample and calculate appropriate average and standard deviation values. The slope obtained for the Blk Wells (sample without cells) should be subtracted from all test values.

There are three available options for measuring fluorescence:
1. Standard fluorescence intensity measurement
2. Time-resolved fluorescence (TR-F) measurement
3. Ratiometric TR-F measurement (subsequent Lifetime calculation)

The Phosphorescent Oxygen Probe can be measured with standard fluorescence intensity or TR-F measurements, using monochromator or filter based platereaders. TR-F measurement reduces non-

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specific background and increases probe sensitivity, offering a more stable reading and wider dynamic range than measuring fluorescence intensity. Ratiometric TR-F measurement can be used to maximize dynamic range and assay performance.

1. Standard Measurement

Optimal wavelengths are 380 nm for excitation and 650 nm for emission. NOTE: This option can often result in a lower signal to background. Time resolved measurements be performed to improve signal to background.

2. TR-F Measurement Optimal wavelengths are 380 nm for excitation and 650 nm for emission with a recommended delay time of 30 µs.

3. Ratiometric TR-F (Lifetime) Measurement Ratiometric TR-F allows for the calculation of lifetime using dual time resolved measurements. In this mode, two separate time resolved readings (W1 and W2) are taken. From these values, a lifetime is calculated using the equation below. NOTE: For accurate calculation of lifetime, ensure that gain values for W1 and W2 are identical.

Lifetime Calculation: Use the dual intensity readings and the following transformation to calculate the corresponding Lifetime (µs):

Lifetime (µs) [τ] = (70-30)/ln(W1/W2)

Where W1 and W2 represent window 1 and 2, respectively, for the measured intensity readings at each time point, and 70 and 30 represent the delay time of W2 and W1, respectively. This provides Lifetime values in µs at each measurement.