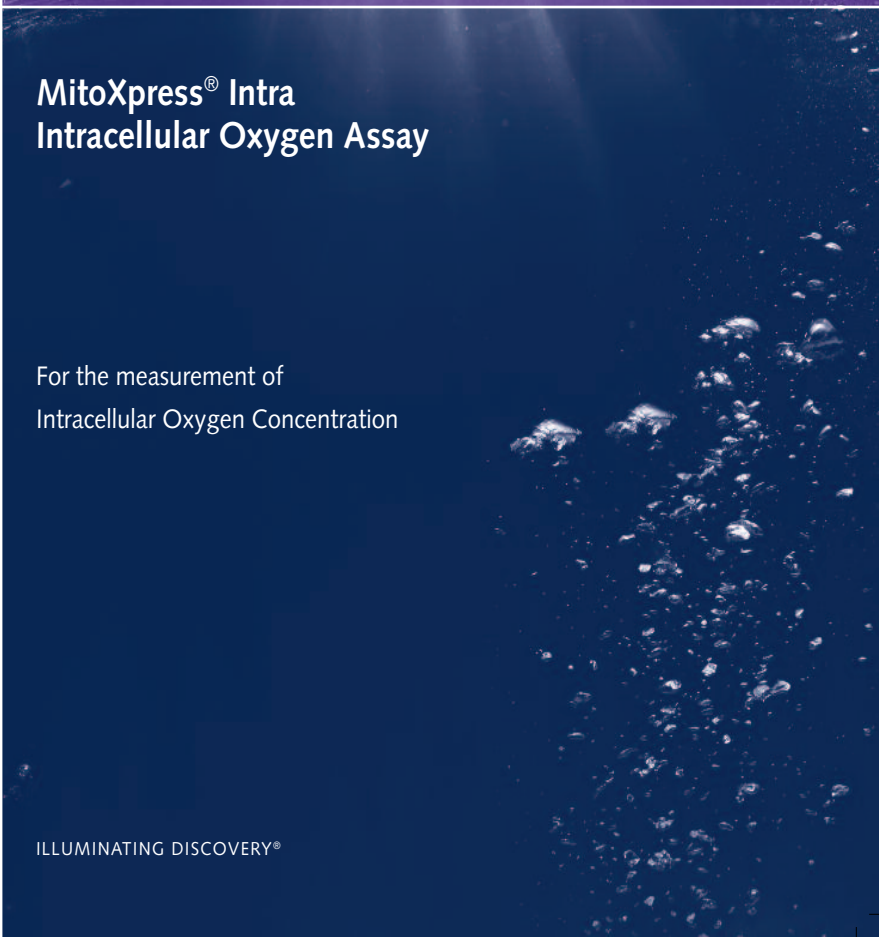
LUXCEL® BIOSCIENCES

MitoXpress® Intra
Intracellular Oxygen Assay

For the measurement of
Intracellular Oxygen Concentration



ILLUMINATING DISCOVERY®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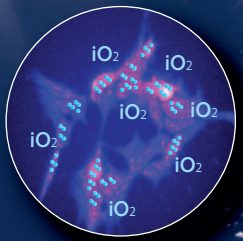
Intracellular Oxygen Assay



For the measurement of
Intracellular Oxygen Concentration
For use with:

- Cell lines
- Stem-cell derived cell types
- Primary cells
- 2D cultures, 3D cultures and spheroids

UM-03 V.1.1

TABLE OF CONTENTS

General Information

MATERIALS SUPPLIED

Assay kit will arrive at room temperature. For best results store as indicated below.

Cat No.	Item	96 well Quantity / Size	Storage
MX-300	MitoXpress® Intra reagent	1 vial	+4°C

STORAGE AND STABILITY

The MitoXpress® Intra Xtra reagent should be stored as follows:

- Dry material between +2 to +8°C (see Use Before date on vial).
- Product reconstituted in sterile water can be stored in the dark between +2 to +8°C, for use within 3 weeks **(DO NOT FREEZE)**.

ADDITIONAL ITEMS REQUIRED

- Fluorescence plate reader, with suitable filter and plate temperature control.
- 96-well (black wall) clear bottom TC+ plates or standard PS plates for cell culture.

OPTIONAL ITEMS NOT SUPPLIED

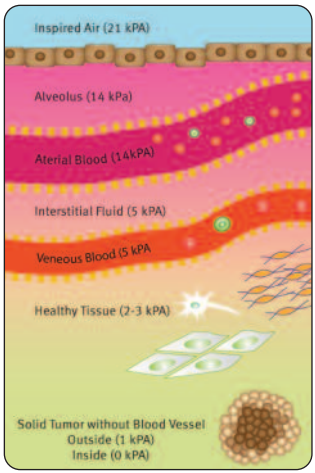
- Plate block heater for plate preparation
- Atmospheric Control Module or Workstation to control CO_2 / O_2 environment

SUPPORT

- Visit our website www.luxcel.com

DESCRIPTION

Oxygen availability significantly influences cell physiology, signal transduction and cellular response to drug treatment. However, in the majority of *in vitro* studies, cells are cultured at ambient oxygen despite the fact that it represents a hyperoxic state for most cell types. The assumption that cells experience this ambient condition also ignores the significant oxygen gradient that can exist between the atmosphere and respiring cells.



To facilitate the convenient investigation and quantification of intracellular oxygen concentration (oxygenation), Luxcel Biosciences has developed the MitoXpress® Intra - Intracellular Oxygen Assay, based on a proprietary O_2-sensitive cell-penetrating nanoparticle probe. The MitoXpress®-Intra fluorophore, is chemically stable and inert, and is taken up by cells during an overnight loading period. Oxygen quenches the phosphorescent emission of the probe, such that measured signal (Ex/Em: 380nm/650nm) is proportional to intracellular oxygen concentration ([iO_2]), thereby allowing real-time monitoring of intracellular oxygen concentration in conventional 2D culture as well as a wide range of 3D systems, including Matrigel®, RAFT™, microtissues, Alvetex®, Mimetix® and other scaffold systems.

MitoXpress® Intra provides a unique tool to quantitatively monitor the oxygen concentration that cells in culture are ACTUALLY experiencing. Additionally, where the experimental objective is to monitor cell physiology under defined O_2 conditions, MitoXpress® Intra provides the ideal tool to identity the appropriate environmental O_2 to achieve this desired cellular O_2 concentration as it accounts for the significant impact cell respiration can have on intracellular O_2 concentration. Luxcel's flexible plate reader format, also allows multiparametric or multiplex combinations with related Luxcel products, as well as other commonly used reagents. For example, MitoXpress® Intra in combination with the Luxcel pH-Xtra™ - Glycolysis Assay (Cat No. PH-200) allows a detailed analysis of the interplay between O_2 availability and concomitant alterations in the balance between aerobic and glycolytic ATP production; investigations that are particularly relevant in the study of tumor metabolism.

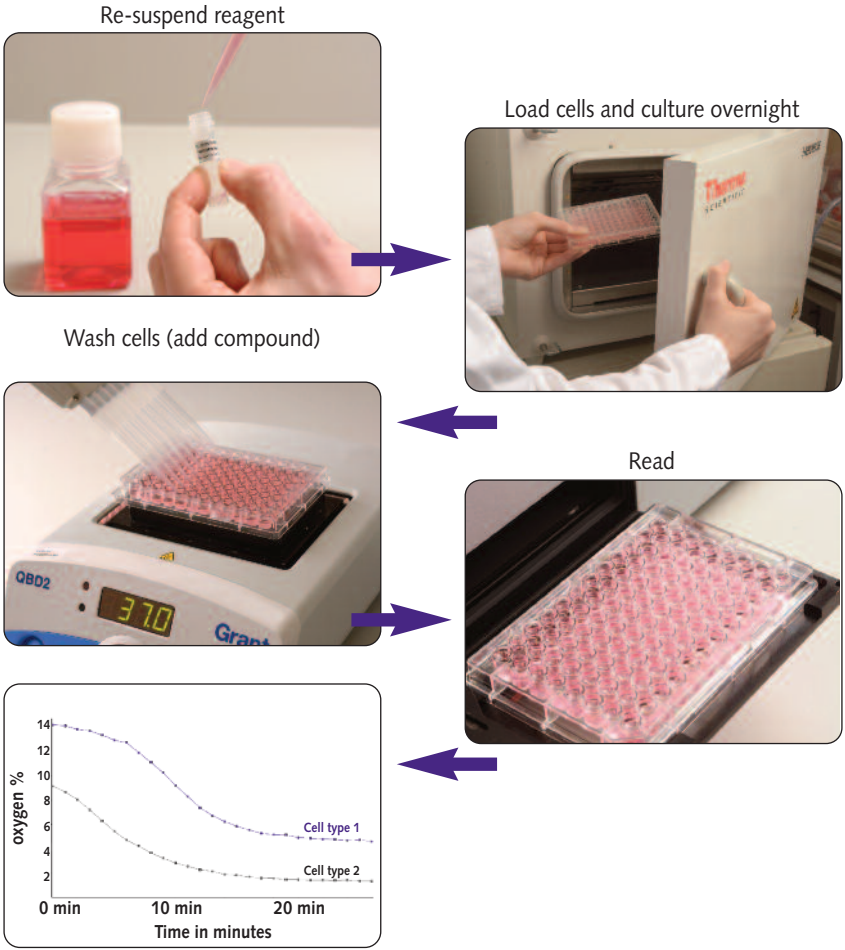


Figure 1: Flow diagram showing preparation and use of MitoXpress® Intra - Intracellular Oxygen Assay

PLATE READER SET-UP

MEASUREMENT PARAMETERS

MitoXpress® Intra reagent is a chemically stable and inert, nanoparticulate, oxygen-sensing fluorophore.

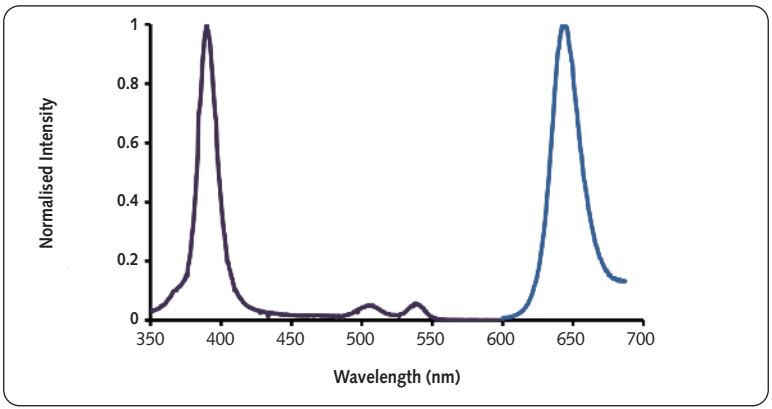


Figure 2: Excitation and Emission spectra of MitoXpress® Intra, showing normalized excitation (Ex 360-400nm; Peak 380nm) and emission (Em 630-670nm; Peak 650nm).

INSTRUMENTS AND SETTINGS

Two fluorescence modes can be successfully used with the MitoXpress®-Intra Intracellular Oxygen Assay.

1 **Standard:** Time-resolved fluorescence measurement (TR-F)
2 **Advanced:** Dual-read Ratiometric TR-F measurement (Lifetime calculation)

NOTE: MitoXpress® Intra - Intracellular Oxygen Assay may also be used semi-quantitatively in non-TR-F intensity mode, although we recommend running the described Signal Optimisation protocol and optimising cell seeding density.

NOTE: Further details, including instrument, filter selection and measurement settings can be found in Appendix A: Instrument Settings.

SIGNAL OPTIMISATION - *recommended for first time users*

NOTE: Use a plate block heater for plate preparation and pre-warm plate reader to measurement temperature.

STEP 1: Reconstitute contents of the MitoXpress® Intra reagent vial in 1mL of sterile water, gently aspirating 3-4 times. *NOTE: Reconstituted stock can be stored in the dark between +2 to +4°C for up to 3 weeks* ***(DO NOT FREEZE).***

STEP 2: Prepare 8 wells of a 96-well plate, by adding 150µL pre-warmed culture medium to each well (A1-A4, B1-B4).

STEP 3: Add 2.5µL of reconstituted MitoXpress® Intra reagent to 4 replicate wells (A1-A4) and 2.5µL culture medium to the remaining replicate wells (B1-B4).

STEP 4: Read plate immediately in a fluorescence plate reader over 30 minutes (read every 2-3 minutes).

STEP 5: Examine Signal Control well (A1-A4) and Blank Control well (B1-B4) signals and calculate signal-to-blank (S:B) ratio using the 30 minute read values. *NOTE: For dual read TR-F, calculate S:B for each measurement window.*

For most fluorescence TR-F plate readers, set up according to Appendix A - Instrument Settings, MitoXpress® Intra reagent should return a S:B ≥ 8. See Appendix D - Troubleshooting.

	1	2	3	4
A	Media + MitoXpress® Intra	Media + MitoXpress® Intra	Media + MitoXpress® Intra	Media + MitoXpress® Intra
B	Media	Media	Media	Media

Performing the Intracellular Oxygen Assay

CELL CULTURE AND PLATING

NOTE: Always leave two wells (H11 and H12) free from the addition of MitoXpress® Intra reagent, as Blank Controls.

STEP 1: Count cells and adjust to the desired plating density in culture medium, (typically 30,000 – 80,000 cells per well for 2D cultures, see Appendix C - Explanatory Notes for information on 3D plating). To eliminate possible plate effects relating to evaporation and cell growth we recommend adding 200µL of culture medium only (without cells) to the outer wells of 96 well plate (columns 1 and 12, rows A and H). Return the plate to culture overnight (typically > 14h).

MITOXPRESS® INTRA REAGENT LOADING

STEP 2: Reconstitute the contents of the MitoXpress® Intra vial in 1mL of sterile water aspirating 3-4 times. Dilute 1 in 10 in culture media to provide sufficient volume to load the required number of wells at 150µL per well, then warm to measurement temperature (typically 37°C).

STEP 3: After overnight culture (Step 1), place the 96-well plate containing cells on the plate block heater equilibrated at measurement temperature. Remove spent media (~200µL) from each well using an aspirator being careful not to dislodge cells from the base of the wells.

NOTE: Do not allow cells to dry out during aspiration.

STEP 4: Using a multichannel or repeater pipette add 100 µL of the pre-warmed MitoXpress® Intra stock (from Step 2), to each well being careful not to dislodge cells and return the plate to culture overnight culture (typically > 14h, see Appendix C - Explanatory Notes for alternative loading options).

Figure3: Reconstitution of MitoXpress® Intra reagent

TYPICAL ASSAY

STEP 5: Place ~25mL of culture media in a 50mL plastic tube and warm to measurement temperature (37°C). For longer term measurements (>2h), we recommend the use of a HEPES buffer. See Appendix C - Explanatory Notes for more information.

STEP 6: Warm instrument to 37°C and prepare a kinetic measurement protocol with the correct instrument settings to read the desired wells at 2-3 minute intervals over the desired duration (see Appendix A: Instrument Settings).

STEP 7: With the plate on a plate block heater, wash cells by removing spent media using an aspirator, and using a multichannel or repeater pipette add 100µL of the prewarmed measurement buffer to each well.

NOTE: Take care not to dislodge cells from the base of the wells.

STEP 8: Repeat wash step and finally add 150µL of pre-warmed measurement buffer to each test well (and to designated Blank wells). The Blank wells are required for the proper Blank correction of the measured fluorescence signal.

NOTE: Plate preparation time should be kept to a minimum.

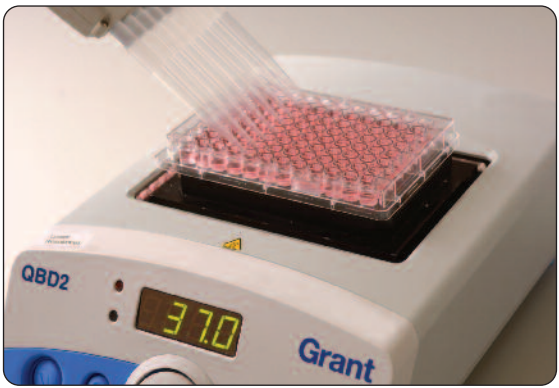


Figure 4: Removing loading media prior to measurement

STEP 9: Insert plate into the fluorescence plate reader and commence kinetic reading. Measure baseline signal for a minimum of 20 minutes to ensure sample temperature equilibration.

ADDING TEST COMPOUND**:** For manual compound addition, pause reading, eject the plate from the reader and quickly add test compound to each well. Re-insert the plate into the plate reader and recommence the kinetic measurement. If available, plate reader injectors can also be used. After responses are observed and oxygen levels have stabilised, further compound additions can be made.

MODULATING OXYGEN ENVIRONMENT: To assess the impact of altered ambient oxygen concentration using an atmospheric control module, after baseline signal has stabilised, alter ambient oxygen concentration as per manufacturer's instructions.

STEP 10: When measurement cycle is complete, remove plate from instrument and save data to file.

STEP 11: Plot the blank-corrected MitoXpress® Intra Intensity or Lifetime values versus time. Results can be further transposed into O_2 scale as described in detail Appendix B - Data Processing and Analysis. Data analysis templates are available from some plate reader manufacturers, specifically configured to automate the analysis of the MitoXpress® Intra - Intracellular Oxygen Assay.

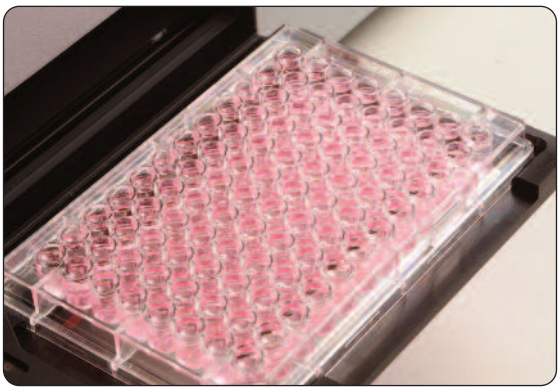


Figure 5: Reading the assay plate

OPTIONAL CONTROLS

SIGNAL CONTROLS:

Negative Signal Control: Leave 2 wells without cells for use as a Negative Signal Control. Add 150µL of fresh culture media + 2.5µL of reconstituted MitoXpress® Intra reagent to each well (only for use when measuring in dual-read TR-F ratiometric mode).

Positive Signal Control: Leave 2 wells without cells for use as a Positive Signal Control. Add 150µL of fresh culture media + 2.5µL reconstituted MitoXpress® Intra reagent and 10µL of (1mg/mL) Glucose Oxidase stock solution (prepared in water) to each well. (If measuring in intensity mode, add Glucose oxidase to wells containing MitoXpress® Intra loaded cells).

BIOLOGICAL CONTROLS:

Negative Biological Controls: To 2 wells containing cells, add 1µL of an Antimycin A stock solution (150µM in DMSO). Antimycin blocks the electron transport chain (ETC) thereby removing the influence of cellular O_2 consumption on iO_2, typically seen as an increase in iO_2.

Positive Biological Control: To 2 wells containing cells, add FCCP to a final concentration of ~2µM. FCCP uncouples respiration thereby increasing cellular O_2 consumption resulting in a decrease in iO_2.

NOTE: As FCCP exhibits a strong bell shaped dose response, a serial dilution should be run of each new cell type to ensure the optimum concentration is used.

ANALYSIS

Data analysis typically uses MitoXpress® Intra Lifetime or Intensity values plotted against time. Results can be further transposed into O_2 scale for further biological insight; data processing steps are described in detail in Appendix B: Data Processing and Analysis. Data analysis templates are available from BMG Labtech and BioTek, specifically configured to automate the analysis of the MitoXpress® Intra – Intracellular Oxygen Assay with their plate readers. *NOTE: We recommend that all first time users perform a Signal Optimisation test, as described.*

ASSESSING INTRACELLULAR OXYGEN CONCENTRATION

Data in O_2 scale can be generated using the conversions detailed in Appendix B - Data Processing and Analysis. Intracellular oxygen concentration can then be plotted against time to assess changes in cellular oxygenation allowing differences between ambient and intracellular O_2 to be determined. For example, HEK293T cultured in 2D and measured at ambient oxygen show an intracellular O_2 level of ~14% (Fig. 6). Reducing instrument O_2 to 6% causes cellular oxygenation to drop to ~2% thereby quantifying the effect of respiratory activity on intracellular O_2.

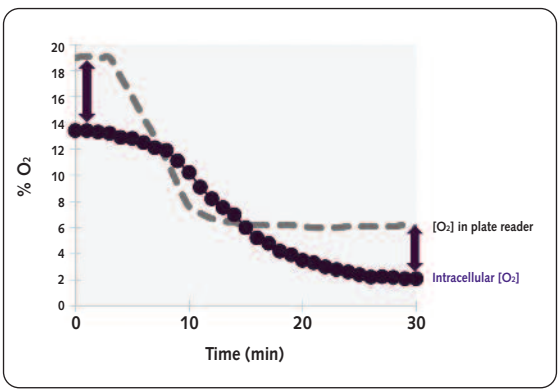


Figure 6. Measuring HEK293T cell oxygenation. MitoXpress® Intra measurement and O_2 modulations performed using a CLARIOstar equipped with an ACU module (BMG Labtech). Instrument O_2 (- - -) and its impact on cellular oxygenation (●) are presented.

IMPACT OF CELL METABOLISM ON OXYGENATION

Cellular oxygenation can be significantly affected by alterations in cell metabolism and these biological processes can be investigated in detail using the MitoXpress® Intra – Intracellular Oxygen Assay. An example is presented in Fig 7 whereby iPS derived cardiomyocytes measured at ambient O_2 (~21%), experience an intracellular O_2 of 14% under resting conditions. If the contribution of cellular respiration is removed through the addition of the electron transport chain (ETC) inhibitor Antimycin, intracellular O_2 slowly returns to ambient levels as the cells cease to consume O_2. If cardiomyocyte beat rate is increased through addition of the β-adrenoreceptor agonist isoproterenol, the resulting increase in ATP demand and O_2 consumption further reduces intracellular O_2, with values of ~6% observed for >15 min. This illustrates the significant O_2 depletion that cells can experience despite being measured at ambient O_2.

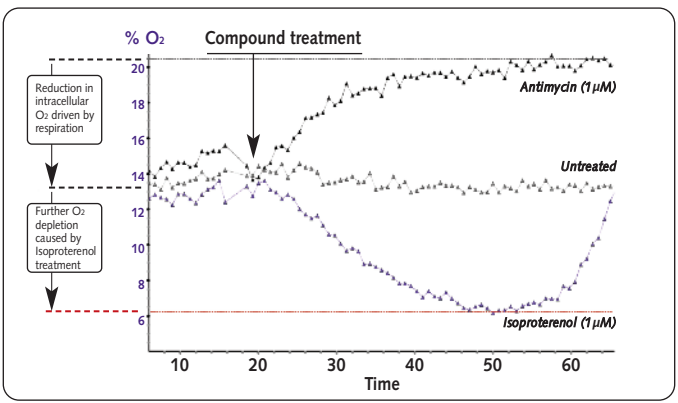


Figure 7. Measuring the impact of cell metabolism on iPS-derived cardiomyocyte oxygenation. During measurement, cells are treated with the ETC inhibitor Antimycin and the β-adrenoreceptor agonist isoproterenol.

OXYGEN SCALE CALIBRATION

A default calibration function has been generated to facilitate the conversion of MitoXpress® Intra Lifetime values into O_2 scale. This conversion is described in detail in Appendix B - Data Processing and Analysis and works well across multiple instruments and cell types. If required, a bespoke calibration function specific to a particular cell type, measurement temperature or measurement conditions can be generated. This requires access to a plate reader with an atmospheric control. An example is presented in Fig 8.

Cells are loaded with MitoXpress® Intra reagent as described and, prior to measurement, treated with the ETC inhibitor Antimycin to remove the influence of cell respiration on intracellular O_2. Measurement begins at atmospheric O_2 (room air) and, using instrument atmospheric control, O_2 is reduced in a stepwise manner (typically ~20, 15, 10, 7.5, 5, 2.5, 1% O_2). Zero values are generated using glucose oxidase as described in the 'Optional Controls' section. Lifetime values are then plotted against applied [O_2] and a first order exponential fit applied to generate a calibration function (Fig. 8). *NOTE: Calibrations are temperature specific and should be conducted at the desired measurement temperature.*

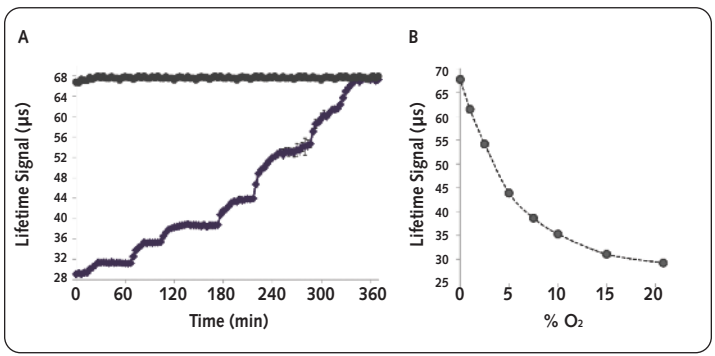


Figure 8: Sample Calibration Data. A) MitoXpress® - Intra Lifetime profiles measured at decreasing [O_2] (purple line) with parallel glucose oxidase treatment to achieve 0% O_2 (grey line). B) The relationship between probe lifetime (τ) and applied [O_2]. B) Applying a first order exponential fit generates a calibration function of $O_2\% = A1 \times Exp(-\tau / t1)$. Example: $O_2\% = 659.3 \times Exp(-\tau / 8.475)$. For additional detail see Appendix B - Data Processing and Analysis.

APPENDIX A - INSTRUMENT SETTINGS

Two fluorescence modes can be successfully used to measure MitoXpress® Intra reagent. Both use Time Resolved Fluorescence (TR-F) which reduces non-specific background and increases probe sensitivity. These measurement modes are outlined below and recommended instrument settings are listed in Table 1.

Standard: Single TR-F Measurement
Single TR-F measurement records probe signal after a defined delay, thereby reducing Blank signals and improving measurement performance. The optimal delay time is ~30µs with a measurement window (integration time) of 100µs. *NOTE: MitoXpress® Intra should return a S:B ≥ 8, values ≥10 are typical. Better S:B is achieved with filter-based optics.*

Advanced: Dual-Read TR-F Measurement (Lifetime)
Optimal performance is achieved using dual-read TR-F (Fig. 9) whereby two intensity measurements are taken sequentially. In combination with the Lifetime calculation detailed in Appendix B, this ratiometric measurement approach monitors the rate of MitoXpress®-Intra fluorescence decay, providing a more robust measurement of oxygen concentration. Optimal dual-delay and window times are: **Read 1:** 30µs delay (D1), 30µs measurement time (W1), **Read 2:** 70µs delay (D2), 30µs measurement time (W2).

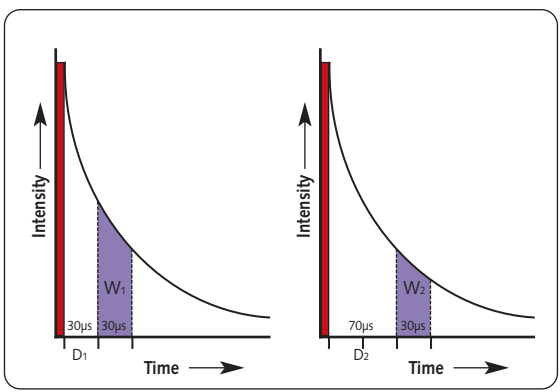


Figure 9: Illustrating dual-read TR-F measurement.

TABLE 1: RECOMMENDED INSTRUMENT SETTINGS

Instrument	Optical Configuration	Integ 1 (D_1 / W_1) Integ 2 (D_2 / W_2)	Optimum Mode*	Ex (nm) Em (nm)
BioTek: Cytation 3 / 5	Filter-based Top or bottom read	30 / 30µs 70 / 30µs	Dual read TR-F (Lifetime)	Ex 380 ± 20nm Em 645 ± 15nm
BioTek: Synergy H1 / Neo / 2	Filter-based Top or bottom read	30 / 30µs 70 / 30µs	Dual read TR-F (Lifetime)	Ex 380 ± 20nm Em 645 ± 15nm
BMG Labtech: CLARIOstar	Filter-based Top or bottom read	30 / 30µs 70 / 30µs	Dual-read TR-F (Lifetime)	Ex 340 ± 50nm (TR-EX) Em 665 ± 50nm or Em 645 ± 10nm
BMG Labtech: FLUOstar Omega / POLARstar Omega	Filter-based Top or bottom read	30 / 30µs 70 / 30µs	Dual-read TR-F (Lifetime)	Ex 340 ± 50nm (TR-EXL) Em 655 ± 25nm (BP-655)
Perkin Elmer: VICTOR series / X4 / X5	Filter-based Top read	30 / 30µs 70 / 30µs	Dual read TR-F (Lifetime)	Ex 340 ± 40nm (D340) Em 642 ± 10nm (D642)
Tecan: Infinite M1000Pro / F200Pro	Monochromator / Filter-based Top or bottom read	30 / 30µs 70 / 30µs	Dual read TR-F (Lifetime)	Ex 380 ± 20nm Em 650 ± 20nm or Em 670 ± 40nm
Tecan: Spark 10M/ 20M	Filter-base / Top or bottom read	30 / 30µs 70 / 30µs	Dual read TR-F (Lifetime)	Ex 380 ± 20nm Em 650 ± 20nm or Em 670 ± 40nm
BioTek: Synergy HTm / Mx	Monochromator / Filter - based Top or bottom read	30 / 100µs n/a	TR-F	Ex 380 ± 20nm Ex 650 ± 20nm
BMG Labtech: PHERAstar FS / FSX	Filter - based Top or bottom read	40 / 100µs n/a	TR-F	Ex 337 (HTR-F module) Ex 655 (HTR-F module)
Tecan: Infinite M200Pro / Safire / Genios Pro	Monochromator / Filter-based Top or bottom read	30 / 100µs n/a	TR-F	Ex 380 ± 20nm Em 650 ± 20nm
Perkin Elmer: Envision / Enspire	Monochromator / Filter based Top read	40 / 100µs n/a	TR-F	Ex 340 ± 40nm (D340) Em 642 ± 10nm (D642)

Notes: Assay-specifc protocols, templates and notes are available from BMG Labtech, BioTek and Tecan (or TechSupport@luxcel.com)
Choose filter based optical configuration where available. Instruments with recommended Dual read TR-F measurement mode can alternatively be set up using standard TR-F measurement mode if desired.

APPENDIX B - DATA PROCESSING AND ANALYSIS

The following description details how to manually calculate lifetime values from measured intensity values, and how to convert either lifetime or intensity data into oxygen scale. Specifically configured data analysis templates available from BMG Labtech and BioTek perform these calculations automatically.

Lifetime calculations from Dual-Read TR-F Measurements

Lifetime (**τ**) values are calculated using the intensity data from Dual-Read TR-F Measurements as follows:

$$\tau = \frac{40}{ln(W1-W2)}$$

where **τ** represents emission lifetime and W1 and W2 represent signals measured at window 1 and window 2. Lifetime values are in µs units, (range ~29 to ~68µs) and should only be calculated from samples containing MitoXpress® Intra reagent. Lifetime values should not be calculated from Blank wells.

Converting into Oxygen Scale

Converting from Lifetime data

Lifetime data is favoured as the basis for generating data in O_2 scale. Lifetime values as calculated above can be converted in oxygen scale using the following default analytical function:

$$O_2\% = 659.3 \times Exp\ \frac{-\tau}{8.475}$$

NOTE: This function is specific to measurements at 37°C. Customized data analysis templates are available from some plate reader manufacturers, specifically configured to automate the analysis of the MitoXpress® Intra – Intracellular Oxygen Assay. Bespoke analytical functions specific to particular cell types or measurement conditions can be generated if desired and require access to a plate reader with an atmospheric control capabilities. An example is presented in Fig. 8.

Converting from Intensity data

Intensity values can be converted into oxygen scale by including a positive control (glucose oxidase addition) and negative control (Antimycin addition). These controls are described in the optional controls section and should be generated on each test plate. Oxygen concentration (**$[O_2]_t$**) is then calculated using the following equation:

$$[O_2]_t = \frac{[O_2]_a \times I_a \times (I_0 - I_t)}{I_t \times (I_0 \times I_a)}$$

whereby **$[O_2]_a$** is oxygen concentration in air-saturated conditions (typically ~20.9%), **I_0** and **I_a** are MitoXpress®-Intra signals measured in deoxygenated and air-saturated conditions respectively, while **I_t** are the experimental intensity values generated during measurement. *NOTE: If the test plate has equilibrated in an environment containing 5% CO_2 and 95% humidity, ambient O_2 = ~18.6%.*

APPENDIX C – EXPLANATORY NOTES

Cell Plating
Typically cells are plated at a density to achieve full confluence. Plating density and basal metabolic rate will determine the steady-state oxygen concentration at the cell monolayer. When plating 3D cultures, prepare the 3D plate or 3D construct solution (e.g. collagen reaction mix) in advance as per manufacturer's instructions. Cells are typically plated at higher concentrations than used for 2D cultures. Lower plating concentration are typically used with long culture times unless cells are terminally differentiated.

Reagent Loading
If necessary, shorter incubation times can be used in combination with a higher concentrations of MitoXpress® Intra reagent. For example, a 6 hour reagent loading incubation using a 1.5X reagent concentration will yield comparable loading. This may be cell type dependent, so optimisation is recommended if changes to reagent loading conditions are made. Reagent loading can also be performed directly in culture flasks if necessary.

Longer Term Measurements
A HEPES-based DMEM measurement buffer allows longer term measurement (>2h) outside a CO_2 incubator as it maintains pH for a prolonged period, without applied CO_2. This is not necessary if using plate reader models equipped with an atmospheric control module where 5% CO_2 can be maintained within the measurement chamber.

HEPES based DMEM receipe: Dissolve DMEM powder (Sigma Aldrich D5030, powder) in ~ 900mL of sterile water. Add 20 mM HEPES, 1 mM Sodium Pyruvate, 20 mM Glucose, 10% FBS, Pen-Strep (if required). Filter sterilize prior to use.

APPENDIX D – TROUBLE SHOOTING

Extensive literature, including Protocols, Application Notes, Videos, Publications and email technical support is also available through our website www.luxcel.com

GENERAL NOTES AND RECOMMENDATIONS

Storage and Stability: On receipt the MitoXpress® Intra reagent should be stored between +2 to +8°C (see Use Before date on vial). Reconstituted reagent stock can be stored in the dark between +2 to +8°C for 3 weeks **(DO NOT FREEZE)**.

Plate Reader: A fluorescence plate reader capable of measuring in TR-F mode, excitation at 380nm and emission at 650nm, and having plate temperature control is required.

Plates: We recommend 96 black wall / clear bottom TC+ plates, although standard clear wall polystyrene plates for cell culture may also be used. The assay is also compatible with many 3D culture plate systems.

Temperature: We recommend the use of a plate block heater for plate preparation steps, to maintain a temperature of 37°C. Pre-warm the fluorescence plate reader to measurement temperature and ensure that all culture media and stock solutions to be used in the assay are pre-warmed at 37°C prior to use.

Signal Optimisation and Use of Controls: We recommend performing a Signal Optimisation check, especially for first time users.

General Assay Set-Up, Pipetting and Aspirating: Prepare your assay, materials and work space in advance. Take care not to disrupt the cell monolayer (adherent cells) during pipetting and aspirating.

Cell Type and Cell Density: Since the MitoXpress® Intra reagent requires cell loading, measured signal is dependent on both cell confluence and cell loading. Increased cell densities can also result in lower intracellular oxygen concentrations due to increased levels of cellular oxygen consumption.

SIGNAL TO BLANK (S:B) OPTIMISATION

For most fluorescence plate readers, set up according to Appendix A, MitoXpress® Intra should return a signal to blank ratio ≥ 8 Values of >10 are typical. The following options may be helpful to improve S:B if the determine ratio is not as high as expected:

1 Increase Gain (PMT) setting or flash energy
2 Adjust TR-F focal height
3 Repeat without phenol red or serum
4 Repeat as top or bottom-read, respectively
5 Increase the loading concentration of MitoXpress® Intra
6 Contact Instrument Supplier for further options

FREQUENTLY ASKED QUESTIONS:

Q: What do I do if I cannot detect any signal in wells containing cells and MitoXpress® Intra (or I can detect a signal but the slope (rate) appears very low)?
A: Check correct Instrument Settings (Appendix A) - Perform Signal Optimisation - Include GOx control (max signal) - Increase cell density. If tested and not resolved, contact TechSupport@luxcel.com

Q: What do I do if I can detect a signal in wells containing MitoXpress® Intra loaded cells, but the signal level falls initially or is variable from well to well?
A: Check cell seeding and pipetting consistency, increase cell density, ensure plate, instrument and all culture media and stock solutions are pre-warmed at 37°C prior to use, reduce plate preparation times.

NOTE: Some plate readers have inconsistent temperature control. If you suspect this to be the case, consider: – Reduce assay (and equilibration) temperatures to 30°C and avoid outer wells. If tested and not resolved, contact TechSupport@luxcel.com

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