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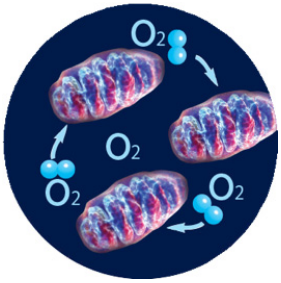
Agilent MitoXpress Xtra

Oxygen Consumption Assay
(HS Method)

For the measurement of Extracellular Oxygen Consumption

For use with:

- Adherent cells
- Suspension cells
- Permeabilized cells
- Isolated mitochondria
- 3D cultures: tissues, spheroids
- RAFT and scaffolds
- Isolated enzymes
- Bacteria, yeasts and molds



Notices

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Contents

1 General Information

Materials Supplied

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

Cat. #	Item	96-well* Quantity/Size	Storage
MX-200-4	MitoXpress Xtra reagent	4 Vials	+4 °C
	HS Mineral oil	4 Dropper bottles/15 mL	Room temp/dark

* May also be used in a 384-well format, with one vial of probe sufficient for ~200 wells

Storage and Stability

The MitoXpress Xtra reagent should be stored as follows:

- Dry material between +2 to +8 °C (see Exp. Date on vial).
- Reconstituted product can be aliquoted and stored at -20 °C. Use within one month (avoid freeze thaw).

Additional Items Required

- Fluorescence plate reader, with suitable filter(s) and plate temperature control.
- Standard clear 96-well TC$^+$ plates OR 96-well black wall clear bottom TC$^+$ plates.

Optional Items Not Supplied

- Repeater pipette
- Plate block heater for plate preparation
- Controls: Glucose Oxidase, Antimycin A

Support

Visit our website **www.agilent.com**.

2 Description

MitoXpress Xtra - Oxygen Consumption Assay (HS Method) is a highly flexible 96- or 384-well fluorescence plate reader-based approach, for the direct, real-time analysis of cellular respiration and mitochondrial function. The easy-to-use MitoXpress Xtra assay allows measurement of extracellular oxygen consumption rates (OCR) with whole cell populations (both adherent and suspension cells), isolated mitochondria, permeabilized cells and a wide range of 3D cultures including: tissues, small organisms, spheroids, scaffolds and matrixes. The assay is also suitable for measurement of isolated enzymes, bacteria, yeasts and molds.

The MitoXpress Xtra reagent is chemically stable and inert, water-soluble and cell impermeable, making it the ideal and scalable mix-and-measure reagent for use in a wide range of cell culture conditions - all measured using a fluorescence plate-reader.

In this assay, MitoXpress Xtra is quenched by O_2, through molecular collision, and thus the amount of fluorescence signal is inversely proportional to the amount of extracellular O_2 in the sample. Rates of oxygen consumption are calculated from the changes in fluorescence signal over time. The reaction is non-destructive and fully reversible (neither MitoXpress Xtra nor O_2 are consumed), facilitating measurement of time courses and drug treatments.

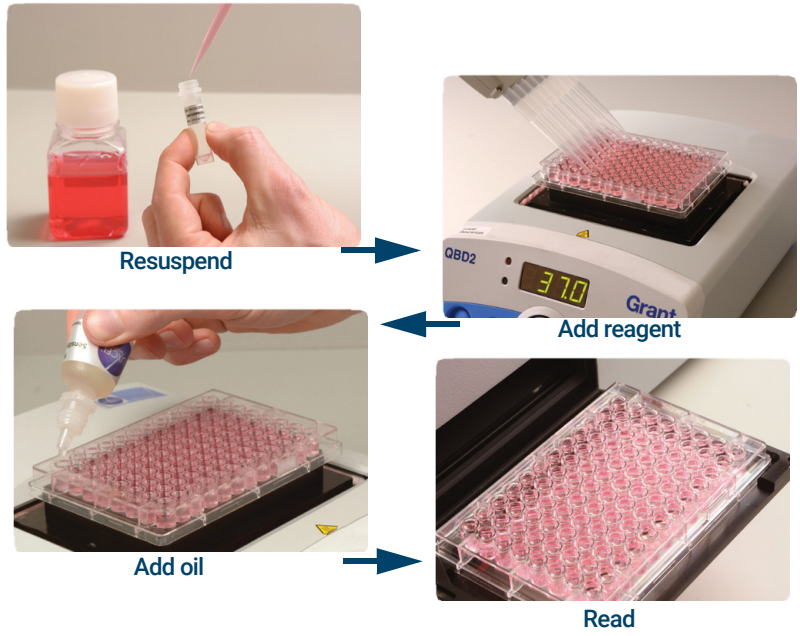

Figure 1. Flow diagram showing preparation and use of MitoXpress Xtra - Oxygen Consumption Assay (HS Method)

3 Plate Reader Set-Up

Measurement Parameters

MitoXpress Xtra reagent is a chemically stable and inert, biopolymer-based, cell impermeable oxygen-sensing fluorophore.

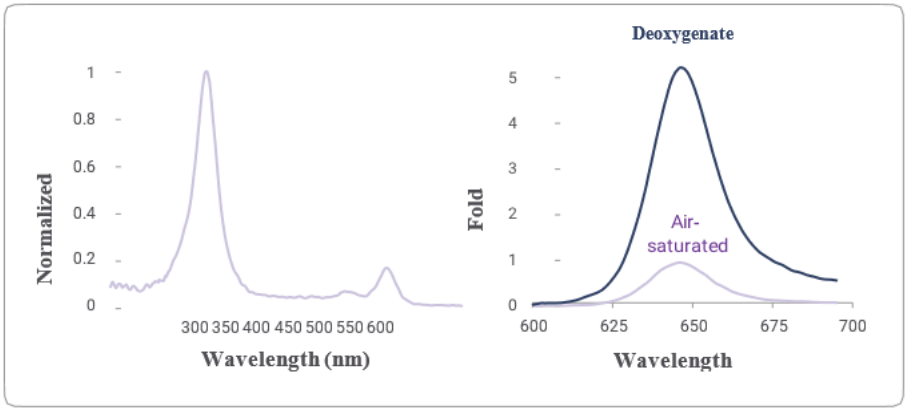

Figure 2. Excitation and Emission spectra of MitoXpress Xtra. Left panel shows normalized excitation (Ex 360-400 nm; Peak 380 nm). Right panel shows emission (Em 630 - 680 nm; Peak 650 nm) in oxygenated and deoxygenated conditions.

Instruments and Settings

Three fluorescence modalities can be successfully used with the MitoXpress Xtra - Oxygen Consumption Assay (HS Method), depending on plate reader type and instrument setup, as follows:

1 **Basic:** Intensity measurement,

2 **Standard:** Time-resolved fluorescence measurement (TR-F), and

3 **Advanced:** Dual-read Ratiometric TR-F measurement (Lifetime calculation).

NOTE

Further details, including instrument, filter selection and measurement settings can be found in "Appendix A - Explanatory Notes" on page 29.

Signal Optimization- Recommended for First Time Users

NOTE **Use a plate block heater for plate preparation and prewarm plate reader to measurement temperature.**

STEP 1: Reconstitute contents of a MitoXpress Xtra vial in 1 mL of water, PBS or culture media, gently aspirating 3-4 times.

NOTE **Reconstituted probe stock can be stored in the dark between +2 to +8 °C for two days or stored as aliquots in water at -20 °C for use within one month (avoid freeze thaw).**

STEP 2: Prepare eight replicate wells of a 96-well plate by adding 90 µL prewarmed culture medium to each well (A1-A4, B1-B4).

STEP 3: Add 10 µL reconstituted MitoXpress Xtra reagent to four of the replicate wells (A1-A4) and 10 µL water, PBS or media to the remaining replicates wells (B1-B4).

STEP 4: Promptly add two drops (or 100 µL) prewarmed HS Mineral Oil to all eight replicate wells, taking care to avoid air bubbles. See **"Appendix B - HS Mineral Oil Pipetting Tips"** on page 35

STEP 5: Read plate immediately in a fluorescence plate reader over 30 minutes (read every 2-3 minutes).

STEP 6: Examine Signal Control well (A1-A4) and Blank Control well (B1-B4) readings, and calculate S:B ratio using the last reading at 30 minutes.

NOTE **For dual read TR-F, calculate S:B for each measurement window.**

For most fluorescence plate readers, set up according to **"Appendix A - Explanatory Notes"** on page 29, MitoXpress Xtra should return a S:B ≥ 3. Higher readings are expected with TR-F and dual read TR-F measurement. See also **"Appendix C - Troubleshooting"** on page 37.

	1	2	3	4
A	Media + MitoXpress Xtra + Oil	Media + MitoXpress Xtra + Oil	Media + MitoXpress Xtra + Oil	Media + MitoXpress Xtra + Oil
B	Media + Oil	Media + Oil	Media + Oil	Media + Oil

3 Plate Reader Set-Up

Signal Optimization- Recommended for First Time Users

4 Performing the Oxygen Consumption Assay

Cell Culture and Plating

NOTE

Always leave at least six wells per 96-well plate free from the addition of cells as Blank and Signal control wells. See section "Recommended controls (optional):" on page 21 for further details.

- For Adherent cells, seed cells in a 96-well plate in 200 µL culture medium. Incubate overnight in a CO_2 incubator at 37 °C.
- For Suspension cells, seed on the day of assay in 100 µL culture medium.

NOTE

Required seeding densities are cell type dependent. We recommend performing a cell seeding density titration experiment to determine the optimal cell number per well, starting with a high cell density (typically 60,000 - 80,000 cells/well for adherent cells and 5 - 6 x 105/well for suspension cells in a 96- well plate). See "Appendix C - Troubleshooting" on page 37for further details.

Pre-Assay Preparation

- Reconstitute the contents of a MitoXpress Xtra vial in 1 mL of water, PBS or culture media, gently aspirating 3-4 times (**Figure 3**). NOTE: Reconstituted probe stock can be stored in the dark between +2 to +8 °C for two days or stored as aliquots in water at -20 °C for use within one month (avoid freeze thaw).
- Prepare test compounds, controls and dilutions as desired.

Figure 3. Reconstruction of MitoXpress Xtra vial

NOTE

We recommend that all culture media and stock solutions to be used in the assay are prewarmed at 37 °C prior to use. Use a plate block heater for plate preparation and prewarm the fluorescence plate reader to measurement temperature.

Recommended controls (optional):

Blank control: Leave two or three wells free from the addition of cells for use as Blank control. Add in total 100 µL of fresh culture media to each well as described in Steps 1 and 2 in the **"Typical Assay"** on page 23. Do not add MitoXpress Xtra reagent to these wells.

Recommended controls (optional):

Cell-free negative control: Leave two or three wells free from the addition of cells for use as Cell-free negative control. Add 90 µL of fresh culture media + 10 µL of reconstituted MitoXpress Xtra reagent to each well as described in Steps 1 and 2 in the **"Typical Assay"** on page 23.

Cell-free positive control: Leave two or three wells free from the addition of cells for use as Cell-free positive controls. Add 90 µL of fresh culture media + 10 µL of (1 mg/mL) Glucose Oxidase stock solution (in water) + 10 µL reconstituted MitoXpress Xtra reagent to each well. If plate reader settings are correct, these wells will show a rapid increase in Fluorescence Intensity/Lifetime as sign for oxygen consumption caused by Glucose Oxidase activity.

Cell-based negative control: To two or three wells containing cells, add 1 µL of (100 µM) Antimycin A stock solution (in DMSO) + 10 µL reconstituted MitoXpress Xtra reagent as described in Steps 1 and 2 in the **"Typical Assay"** on page 23. Antimycin A, a Complex III inhibitor, will inhibit oxygen consumption caused by mitochondrial respiration.

Typical Assay

To assess Oxygen Consumption or to investigate the effect of a compound on electron transport chain function (ETC; oxidative phosphorylation), cells are treated immediately prior to measurement.

NOTE **We recommend the use of triplicate wells for each treatment.**

STEP 1: Remove spent culture medium from all assay wells and replace with 90 µL of fresh culture media (**Figure 4** on page 24).

NOTE **We recommend to always use at least two wells without cells as Blank control wells (do not add MitoXpress Xtra reagent into these wells) and at least two additional wells without cells as Cell-free negative control wells (add MitoXpress Xtra reagent into these wells in Step 2). Also consider to include a Cell-free positive control as described in the "Recommended controls (optional):" on page 21. Add 90 µL of fresh culture media to these Control wells also.**

STEP 2: Add 10 µL reconstituted MitoXpress reagent to each well, except those wells for use as Blank Controls. Add 10 µL of fresh culture media to these Blank Control wells.

NOTE **If plating a full 96-well plate of assays, we recommend combining Step 1 and Step 2 by adding the 1 mL of reconstituted MitoXpress Xtra reagent to 10 mL prewarmed fresh culture media and using a multichannel pipette to add 100 µL of MitoXpress Xtra in media stock to each well (Figure 4 on page 24). Add 100 µL of fresh culture media only (no MitoXpress Xtra) to the Blank Control wells.**

STEP 3: Test compound stock or vehicle (typically 1-10 µL) may be added at this point if desired.

NOTE **We recommend keeping the volume of added compound low to minimize any potential effects of solvent vehicle.**

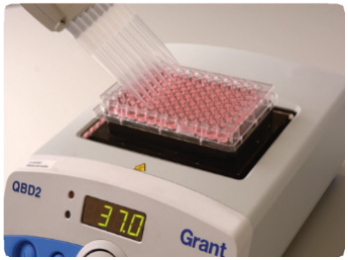

Figure 4. Adding fresh media ± MitoXpress Xtra

STEP 4: Promptly seal each well by adding two drops (or 100 µL) prewarmed HS Mineral Oil, taking care to avoid air bubbles (**Figure 5**).

NOTE

Small variations in the volume of oil (between 90-110 µL) should not adversely affect the readings using MitoXpress Xtra. See also "Appendix B - HS Mineral Oil Pipetting Tips" on page 35.

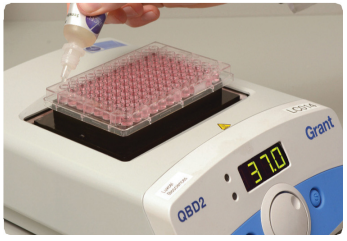

Figure 5. Adding prewarmed HS Mineral Oil

STEP 5: Read the plate immediately in a fluorescence plate reader, with the set-up as described in **"Appendix A - Explanatory Notes"** on page 29 (**Figure 6** on page 24). The plate should be measured kinetically for > 90 minutes. When measurement is completed, remove the plate and save measured data to file.

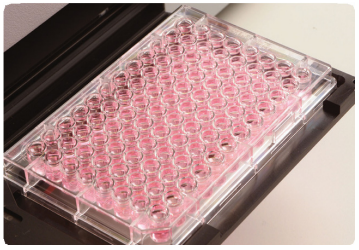

Figure 6. Reading the assay plate

5 Analysis

NOTE **We recommend that all first time users perform a Signal Optimization test as described.**

Assessing Oxygen Consumption

Plot the Blank Control well-corrected MitoXpress Xtra Intensity or Lifetime values versus Time (mins; **Figure 7**). Select the linear portion of each signal profile (avoiding any initial lag or subsequent plateau) and apply linear regression to determine the slope (OCR) and correlation coefficient for each well.

NOTE **This approach is preferable to calculating a slope from averaged profiles.**

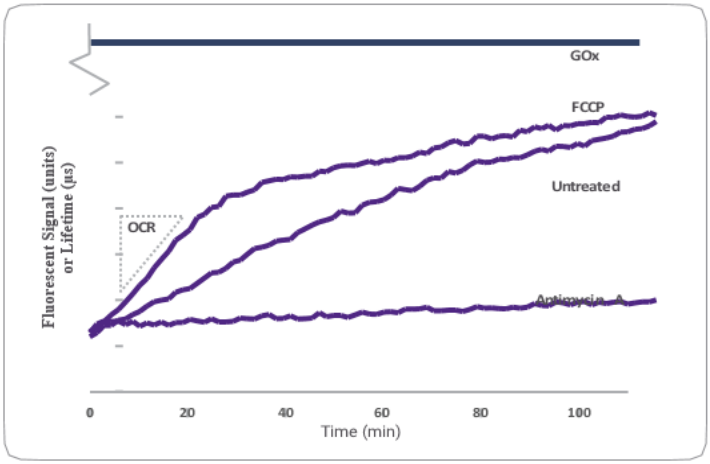

Figure 7. Typical Signal profile (Intensity/TR-F arbitrary units [AU] or Lifetime [µs]) for adherent cells treated with different ETC compounds, including Antimycin A (recommended as a Negative Control). The effect of Glucose Oxidase as a positive Signal Control is illustrated schematically.

NOTE **If using FCCP it is strongly recommended to perform a dose titration, since FCCP exhibits a bell-shaped response.**

Tabulate the slope values for each test sample, calculating appropriate average and standard deviation values across replicate wells. The slope obtained for the Cell-free negative control samples or for the Cell-based negative control samples can be subtracted from all test values if desired.

Protocols, data analysis templates and other technical resources are available for download through our website **www.agilent.com**.

Plotting a Dose Response Curve

To generate a dose response curve, plot the data generated as outlined above against the corresponding compound concentration (**Figure 8**).

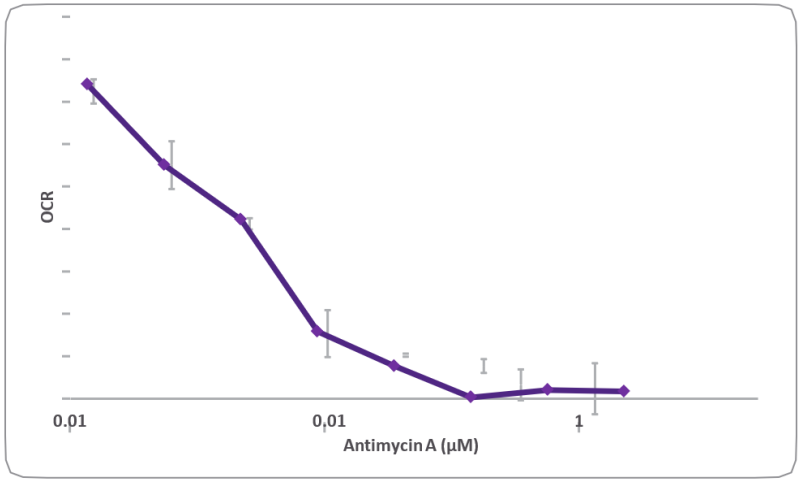

Figure 8. The dose response curve presented here is an example of the data typically produced with this assay. Plotting the calculated oxygen consumption rate (OCR) versus drug concentration demonstrates that Antimycin A causes inhibitory response on cellular respiration.

Measuring Altered Metabolism

Multiparametric (or multiplex) combination of MitoXpress Xtra - Oxygen Consumption Assay (HS Method) together with pH-Xtra - Glycolysis Assay (Cat No. PH-200-4) allows the simultaneous real-time measurement of mitochondrial respiration and glycolysis and analysis of the metabolic phenotype of cells and the shift (flux) between the two pathways under pathological states (**Figure 9**).

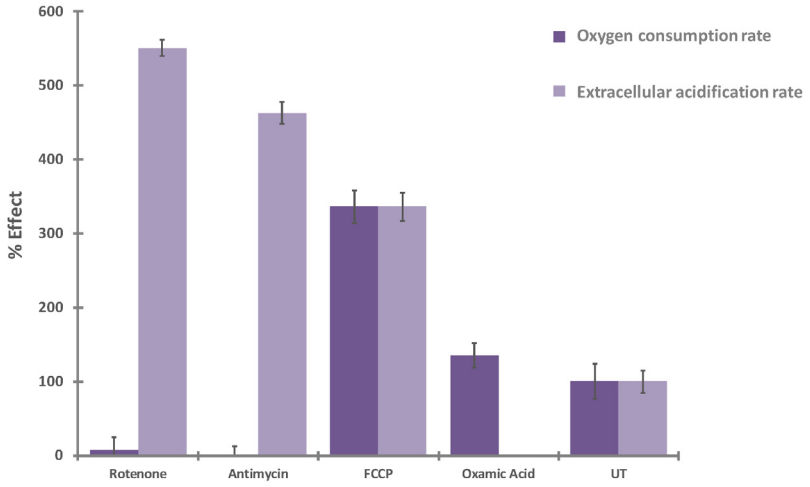

Figure 9. Measuring altered metabolism in HepG2 cells treated with compounds modulating the ETC or inhibiting lactate production, shown as a percentage relative to untreated control cells. Comparative measurements with MitoXpress Xtra and pH-Xtra show the shift between mitochondrial respiration and glycolysis.

Appendix A - Explanatory Notes

Three fluorescence modalities can be successfully used depending on plate reader type and instrument setup.

NOTE **We strongly recommend only using fluorescence plate readers equipped with temperature control. If available, performing TR-F or Dual-Read TR-F measurements is recommended.**

Basic: Intensity Measurement

Measurement of Signal Intensity (sometimes referred to as prompt) provides flexibility to use a very wide range of commonly available fluorescence, monochromator or filter-based plate readers. Optimal wavelengths are 380 nm excitation and 650 nm for emission, with detection Gain parameters (PMT) typically set at medium or high.

NOTE **MitoXpress Xtra should return a S:B ≥ 3**

Standard: TR-F Measurement

Increased levels of performance can be achieved by using time-resolved fluorescence (TR-F). TR-F measurement reduces nonspecific background and increases probe sensitivity. Optimal delay time is ~30 µs and gate (integration) time is 100 µs.

NOTE **MitoXpress Xtra should return a S:B > 3 S:B ~10 are typical.**

Advanced: Dual-Read TR-F (Lifetime)

Optimal performance can be achieved using dual-read TR-F in combination with subsequent ratiometric Lifetime calculation, to maximize dynamic range (**Figure 10** on page 31).

NOTE

MitoXpress Xtra should return a S:B ≥ 3 and S:B up to 60 are possible.

Optimal dual-delay and gate (integration) times:

- Integration window 1: 30 µs delay (D_1), 30 µs measurement time (W_1)
- Integration window 2: 70 µs delay (D_2), 30 µs measurement time (W_2)

Dual-Read TR-F and Lifetime Illustrated

Dual-read TR-F and subsequent Lifetime calculation allows measurement of the rate of fluorescence decay of the MitoXpress Xtra reagent, and can provide measurements of oxygen consumption that are more stable and with a wider dynamic range than measuring Signal Intensity.

NOTE **S:B for Integration window 2 is recommended to be ≥ 10 to allow accurate Lifetime calculation.**

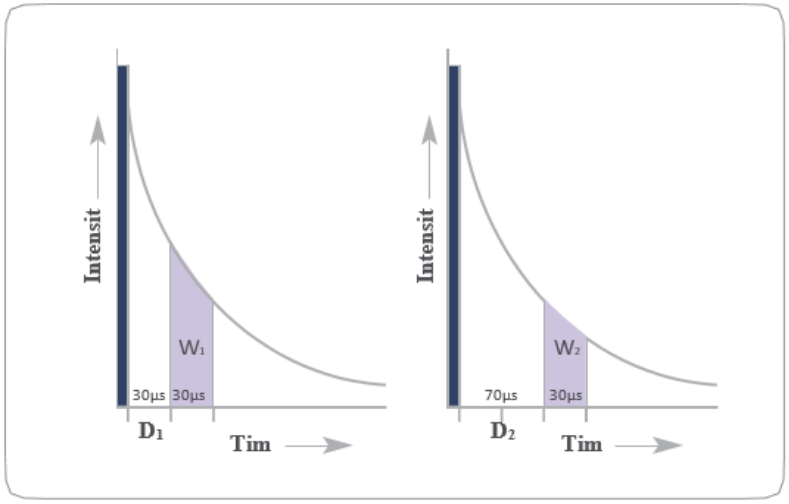

Figure 10. Illustrating dual-read TR-F measurement.

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

Lifetime (µs)[**τ**] $=(D_2-D_1)/\ln(I_{W1}/I_{W2})$

Where I_{W1}/ and I_{W2} represent the two (dual) measurement windows and D_1 and D_2 represent the delay time prior to measurement of W_1 and W_2 respectively. This provides Lifetime values in microsecond units (µs) at each measured time point for each individual sample (**Figure 10**).

NOTE **Lifetime values should be in the range ~22 to ~68 µs, and should only be calculated from samples containing MitoXpress Xtra reagent. Lifetime values should not be calculated from blank wells.**

Table 1 Recommended Instrument and Measurement Settings

Instrument	Optical configuration	Integ 1 (D1/W1) Integ 2 (D2/W2)	Optimum mode	Ex (nm) Em (nm)
BioTek: Cytation 1, 3 or 5 Synergy H1, H4, Neo or Neo2	Filter-based Top or bottom read	30 / 30 µs 70 / 30 µs	Dual read TR-F (Lifetime)	Ex 380 ± 20 nm Em 645 ± 15 nm
BMG Labtech: CLARIOstar	Filter-based Top or bottom read (bottom read preferred where available)	30 / 30 µs 70 / 30 µs	Dual-read TR-F (Lifetime)	Ex 340 ± 50 nm (TR-EX) Em 665 ± 50 nm or Em 645 ± 10 nm with LP-TR Dichroic
FLUOstar Omega POLARstar Omega	Filter-based Top or bottom read	30 / 30 µs 70 / 30 µs	Dual-read TR-F (Lifetime)	Ex 340 ± 50 nm (TR-EXL) Em 655 ± 25 nm (BP-655)
Tecan: Spark 10M or 20M	Filter-based Top read	30 / 30 µs 70 / 30 µs	Dual read TR-F (Lifetime)	Ex 380 ± 20 nm Em 670 ± 40 nm
	Fusion optics Top read	30 / 30 µs 70 / 30 µs	Dual read TR-F (Lifetime)	Ex 380 ± 20 nm (Monochromator) Em 670 ± 40 nm (Filter)
Infinite F200Pro Infinite F Plex Infinite F Nano+	Filter-based Top or bottom read (bottom read preferred where available)	30 / 30 µs 70 / 30 µs	Dual read TR-F (Lifetime)	Ex 380 ± 20 nm Em 670 ± 40 nm
Perkin Elmer: VICTOR series, X4 or X5	Filter-based Top read	30 / 30 µs 70 / 30 µs	Dual read TR-F (Lifetime)	Ex 340 ± 40 nm (D340) Em 642 ± 10 nm (D642)
Molecular Devices: SpectraMax i3x, i3 SpectraMax Paradigm	Filter-based Top or bottom read (bottom read preferred where available)	30/100 µs n/a	TR-F	Ex 370 nm Em 642 ± 10 nm (TRF-EuSa Filter Cartridge)
SpectraMax iD5	Filter-based Top or bottom read (bottom read preferred where available)	30 / 100 µs n/a	TR-F	Ex 350 ± 60 nm Em 642 ± 10 nm
	Monochromator-based Top or bottom read (bottom read preferred where available)	30 / 100 µs n/a	TR-F	Ex 380 ± 15 nm Em 650 ± 25 nm

Table 1 Recommended Instrument and Measurement Settings (continued)

Instrument	Optical configuration	Integ 1 (D1/W1) Integ 2 (D2/W2)	Optimum mode	Ex (nm) Em (nm)
BMG Labtech: PHERAstar FS/FSX	Filter-based Top or bottom read	40 / 100 µs n/a	TR-F	Ex 337 Em 665 (HTRF Module)
BMG Labtech: FLUOstar Optima POLARstar Optima	Filter-based Top or bottom read	30 / 100 µs n/a	TR-F	Ex 340 ± 50 nm (TR-EXL) Em 655 ± 25 nm (BP-655)
Tecan: Infinite M1000Pro Infinite M200Pro Infinite M Plex Infinite M Nano^{+}	Monochromator Top or bottom read	30 / 100 µs n/a	TR-F	Ex 380 ± 9 nm Em 650 ± 20 nm
Safire or Genios Pro	Monochromator / Filter-based Top or bottom read	30 / 100 µs n/a	TR-F	Ex 380 ± 9/15 nm Em 650 ± 20 nm
Perkin Elmer: EnVision	Filter-based Top read	40 / 100 µs n/a	TR-F	Ex 340 ± 60 nm (X340) Em 650 ± 8 nm (M650)
Molecular Devices: SpectraMax M series, SpectraMax Flexstation SpectraMax Gemini SpectraMax i3x, i3, iD3 or iD5	Monochromator Top or bottom read (bottom read preferred where available)	n/a n/a	Intensity (Prompt)	Ex 380 ± 15 nm Em 650 ± 25 nm
SpectraMax Paradigm	Filter-based Bottom read	n/a n/a	Intensity (Prompt)	Ex 380 ± 15 nm Em 650 ± 15 nm (TUNE Cartridge)

NOTE **For up-to-date instrument settings, downloadable protocols and data-analysis tools, please visit www.agilent.com.**

Appendix A - Explanatory Notes

Dual-Read TR-F and Lifetime Illustrated

Appendix B - HS Mineral Oil Pipetting Tips

HS Mineral Oil is provided in an easy to use dropper bottle for convenience, although we recommend a repeater pipette for routine use.

Figure 11. Add oil

- **Dropper bottle:** Invert the prewarmed dropper bottle and apply gentle pressure, just sufficient to prime the oil in the bottle tip. Apply two drops to each well, touching each drop as it is formed to the side of the well to allow it to run down onto the surface of the culture media.

- **Repeater pipette:** Use of a repeater pipette saves time and helps to maintain more precise incubation times. Prepare the repeater syringe tip by trimming ~3-4 mm off the tip at a 45 ° angle. Remove the internal nozzle cap from the dropper bottle and slowly pick up the prewarmed HS Mineral Oil (avoid pipetting up and down, as this can cause bubbles) and dispense 100 µL to each well at an angle of ~45 °, allowing the oil to flow the side of each well. NOTE: Small variations in the volume of HS Mineral Oil (between 90-110 µL) should not adversely affect the readings using MitoXpress Xtra.

Appendix C - Troubleshooting

Protocols, data analysis templates and other technical resources are available for download through our website **www.agilent.com**.

General Notes and Recommendations

Storage and stability: On receipt, the MitoXpress Xtra reagent should be stored between +2 to +8 °C (see Exp. Date on vial). Reconstituted probe stock can be stored in the dark between +2 to +8 °C for two days or stored as aliquots in water at -20 °C for use within one month (avoid freeze thaw).

Plate reader: A fluorescence plate reader capable of measuring excitation at 380 nm and emission at 650 nm, and having plate temperature control is required. Increased levels of performance can be achieved by using time-resolved fluorescence (TR-F), if available. TR-F measurement reduces non-specific background and increases probe sensitivity.

Plates: We recommend 96- or 384-well black wall/clear bottom TC+ plates, although standard clear wall PS plates for cell culture may also be used.

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

Signal optimization and Use of controls: We recommend performing a signal optimization check, especially for first time users, and inclusion of blank and optional additional control wells as described.

Pipetting HS oil: Take care when dispensing the HS Mineral Oil to avoid bubbles. Apply HS Mineral Oil allowing it to run down the inside surface of each well. Do not shake or rapidly aspirate the HS Mineral Oil.

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. Work rapidly once the MitoXpress Xtra reagent has been added, to reduce the potential for assay variability.

Cell type and Cell density: Since the MitoXpress Xtra reagent measures extracellular Oxygen Consumption, the amount of signal change will be directly dependent on the rate of cellular respiration of the cell type being measured. We recommend using as high a cell density per well as practical as a starting point, and reducing cell numbers as required. Not all cell types may consume sufficient oxygen for detection.

Signal to Blank (S:B) Optimization

For most fluorescence plate readers, set up according to **"Appendix A - Explanatory Notes"** on **page 29**, MitoXpress Xtra should return a signal to blank ratio ≥ 3. Higher readings are expected with TR-F and dual read TR-F measurement. The following options may be helpful to improve S:B if the determine ratio is not as high as expected:

- Increase Gain (PMT) setting or flash energy/number.
- Adjust TR-F focal height
- Repeat without phenol red or serum.
- Measure as bottom read as available.
- Increase volume of MitoXpress Xtra (15 µL/well).
- Contact Instrument Supplier or **cellanalysis.support@agilent.com** for further options.

FREQUENTLY ASKED QUESTIONS:

Q: What do I do if I cannot detect any signal in wells containing cells and MitoXpress Xtra (or I can detect a signal but the slope (rate) appears very low)?

A: Check correct Instrument Settings (Appendix A) - Perform Signal Optimization - Include Signal controls - Increase cell density. If tested and not resolved, please visit change to Agilent **www.agilent.com**.

Q: What do I do if I can detect a signal in wells containing cells and MitoXpress Xtra, but the slope (rate) 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 prewarmed at 37 °C prior to use - Reduce plate preparation times. Reduce assay volume to 60 µL.

NOTE

Some plate readers have inconsistent temperature control. If you suspect this to be the case, consider reducing assay and equilibration temperatures to 30 °C and avoiding outer wells. If tested and not resolved, please visit www.agilent.com.

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