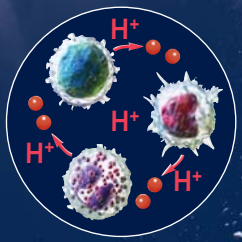
LUXCEL® BIOSCIENCES

pH-Xtra™ Glycolysis Assay



For the measurement of
Extracellular Acidification

ILLUMINATING DISCOVERY®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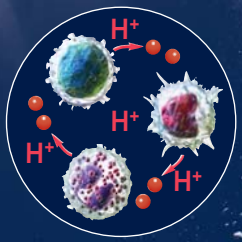
BIOSCIENCES

pH-Xtra™
Glycolysis Assay



For the measurement of
Extracellular Acidification
For use with:

- Adherent cells;
- Suspension cells;
- Permeabilised cells;
- 3D cultures: tissues, spheroids,
- RAFT™ and scaffolds.

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[1.] Quantity / Size	Storage
PH-100	pH-Xtra™ Reagent	1 vial	+4°C
RB-100	Respiration Buffer[2.]	1 tablet	Room Temp

STORAGE AND STABILITY

The pH-Xtra™ reagent should be stored as follows:

- Dry material between +2 to +8°C (see Use Before date on vial).
- Reconstituted pH-Xtra™ reagent can be stored in the dark between +2 to +8°C for several days or reconstituted in water and stored as aliquots at -20°C for use within one month (avoid freeze thaw).

The Respiration Buffer tablet should be stored as follows:

- Dry material at room temperature (see Use Before date on packaging)
- Reconstituted and filter sterilised product can be stored between +2 to +8°C.

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
- 0.22µm sterilization filter, pH meter and acid / base for adjustment

SUPPORT

- Visit our website www.luxcel.com.

[1.] May also be used in a 384-well format, with one vial of reagent sufficient for ~ 200 wells.
[2.] 1mM K-phosphate, 20mM Glucose, 70mM NaCl, 50mM KCl, 0.8mM $MgSO_4$, 2.4mM $CaCl_2$.

DESCRIPTION

The pH-Xtra™ Glycolysis Assay from Luxcel Biosciences is an easy to use, highly flexible 96 or 384-well fluorescence-based approach for the direct, real-time, kinetic analysis of extracellular acidification rates (ECA/ECAR). As lactate production is the main contributor to this acidification, ECA measurements are a convenient and informative measure of cellular glycolytic flux. Such measurements offer an important insight into the central role played by altered glycolytic activity in a wide array of physiological and pathophysiological processes, including cellular adaptation to hypoxia and ischemia, and the development and progression of tumorigensis.

The pH-Xtra™ reagent is chemically stable and inert, water-soluble and cell impermeable. It exhibits a positive signal response (increased signal with increased acidification) across the biological range (pH6-7.5), which, coupled with its spectral and response characteristics, make pH-Xtra™ the ideal choice for flexible, high-throughput assessment of ECA. This performance facilitates sensitive robust microtitre-plate based measurements, thereby overcoming many of the problems associated with the more cumbersome potentiometric pH approach. Rates of extracellular acidification are calculated from changes in fluorescence signal over time and, as the measurement is non-destructive and fully reversible (pH-Xtra™ reagent is not consumed), measurement of time courses and multiple drug treatments are possible.

Luxcel's flexible plate reader format also allows multiparametric or multiplex combinations with Luxcel's other products and with other commonly available reagents, thereby facilitating parallel kinetic measurements of parameters such as ECA, mitochondria membrane potential ($\triangle\Psi m$), O_2 consumption or ROS generation, followed by end-point measure of parameters such as ATP content or cell membrane integrity, all on the same test cells. For example, the combination of Luxcel's MitoXpress® Xtra – Oxygen Consumption Assay (HS Method; Cat No. MX-200) and pH-Xtra™ Glycolysis Assay allows the simultaneous real-time measurement of the interplay between of mitochondrial respiration and glycolysis. This facilitates the determination of a cell's metabolic phenotype and the quantification of perturbations in the balance between glycolysis and oxidative phosphorylation under various stimuli or pathological states.

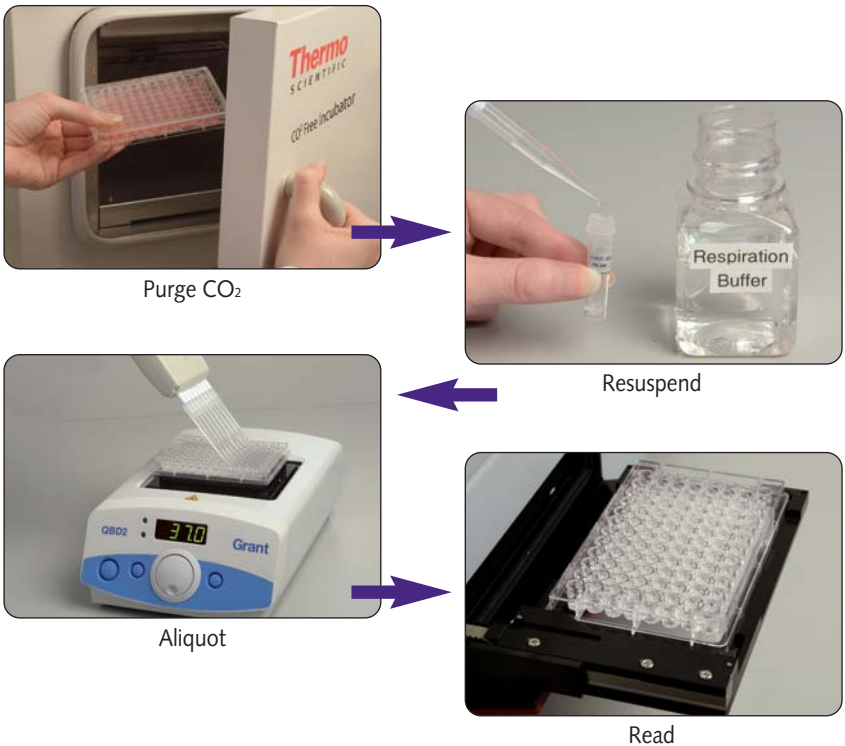


Figure 1: Flow diagram showing preparation and use of pH-Xtra™ Glycolysis Assay

PLATE READER SET-UP

MEASUREMENT PARAMETERS

pH-Xtra™ reagent is a chemically stable and inert, cell impermeable H^+-sensing fluorophore.

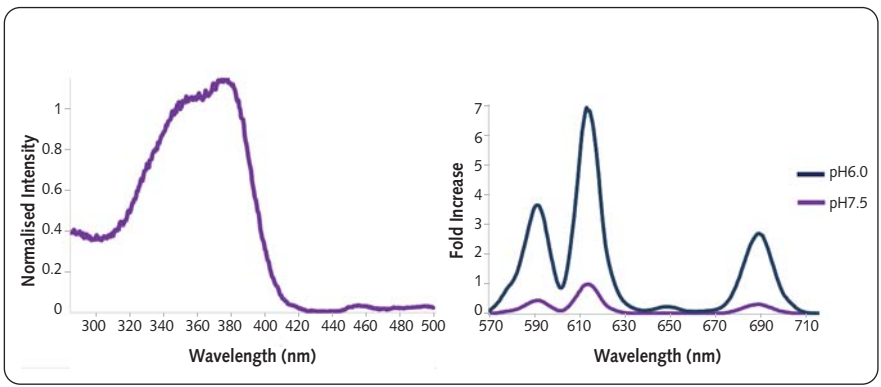


Figure 2: Excitation and Emision spectra of pH-Xtra™. Left panel shows normalized excitation (Ex 340-410nm; Peak 360-380nm). Right panel shows emission maxima (Em 590, 615 and 690nm) fold increase between pH6.0 and pH7.5

INSTRUMENTS AND SETTINGS

Two fluorescence modalities[1.] can be optimally used with the pH-Xtra™ Glycolysis Assay, depending on plate reader type and instrument setup, as follows:

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

[1.] *pH-Xtra™ Glycolysis Assay may also be used in non - TR-F intensity mode on some plate readers, although we recommend running the 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 Respiration Buffer tablet in 50ml of water, warm to assay temperature (37°C), pH adjust to approx. pH7.4 and filter sterilise using a 0.22µm filter. Reconstitute (transparent) contents of the pH-Xtra™ vial in 1ml of Respiration Buffer, gently aspirating 3-4 times. *NOTE: Reconstituted pH-Xtra™ reagent can be stored in the dark between +2 to +8°C for several days or stored as aliquots in water at -20°C for use within one month (avoid freeze thaw).*

STEP 2: Prepare 8 replicate wells of a 96-well plate, by adding 150µl pre-warmed Respiration Buffer to each well (A1-A4, B1-B4).

STEP 3: Add 10µl reconstituted pH-Xtra™ reagent to 4 of the replicate wells (A1-A4) and 10µl Respiration Buffer 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) readings (linear phase) and calculate S:B ratio. *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, pH-Xtra™ should return a S:B ≥3. *NOTE: See also Appendix B – Trouble Shooting.*

	1	2	3	4
A	Respiration Buffer + pH-Xtra™	Respiration Buffer + pH-Xtra™	Respiration Buffer + pH-Xtra™	Respiration Buffer + pH-Xtra™
B	Respiration Buffer	Respiration Buffer	Respiration Buffer	Respiration Buffer

PERFORMING THE GLYCOLYSIS ASSAY

CELL CULTURE AND PLATING

NOTE: Always leave two wells (H11 and H12) free from the addition of pH-Xtra™ reagent, as Blank Controls.

- For Adherent cells, seed cells in a 96-well plate at a density (typically 30,000 – 80,000 cells/well) in 200µl culture medium. *NOTE: For new cell types, we recommend setting up a titration to select the optimum cell seeding density (see Figure 7).*
- For Suspension cells, seed on the day of assay in 150µl culture medium at a density of approx. 250,000-500,000 cells/well.

Visit our website www.luxcel.com for more information on the use of pH-Xtra™ with a range of cell systems.

PRE-ASSAY PREPARATION

NOTE: Where cells are cultured in a CO_2 incubator overnight, it is important to purge the media and plasticware of CO_2 prior to conducting the pH-Xtra™ Glycolysis Assay as residual CO_2 may contribute to acidification. Perform a CO_2 purge, by incubating cells in a CO_2-free incubator at 37°C with 95% humidity, approx. 3 hours prior to performing the Glycolysis Assay measurement.

- Reconstitute Respiration Buffer tablet in 50ml of water, pH adjust to approx. pH7.4 and filter sterilise using a 0.22µm filter. Reconstitute transparent contents of the pH-Xtra™ vial in 1ml of Respiration Buffer, gently aspirating 3-4 times. *NOTE: Reconstituted pH-Xtra™ reagent can be stored in the dark between +2 to +8°C for several 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. Typical controls are oxamic acid (inhibitor; decrease ECA), FCCP (ETC uncoupler; increases ECA) and glucose oxidase (GOx; signal control).

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

Figure 3: Reconstitution of pH-Xtra™ vial

TYPICAL ASSAY

To assess Extracellular Acidification (ECA) or to investigate the effect of a treatment on glycolytic flux, 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 wash cells twice (2x), using 100µl of Respiration Buffer per well for each wash (Figure 4). After removing the second wash, replace with 150µl of fresh Respiration Buffer. *NOTE: We recommend always leaving two wells (H11 and H12) free from the addition of pH-Xtra™ reagent, for use as Blank Controls.* Add 150µl of Respiration Buffer to these Blank Control wells also.

STEP 2: Add 10µl reconstituted pH-Xtra™ reagent to each well, except those wells for use as Blank Controls. Add 10µl of Respiration Buffer to these Blank Control wells.

NOTE: If plating a full 96-well plate of assays, we recommend simplifying Step 1 and 2 by preparing a stock solution containing the 1ml of reconstituted pH-Xtra™ reagent added to 15ml pre-warmed Respiration Buffer, and using a multi-channel pipette to add 150µl of this diluted pH-Xtra™ stock to each well. Add 150µl of Respiration Buffer only (no pH-Xtra™) to each Blank Control well.

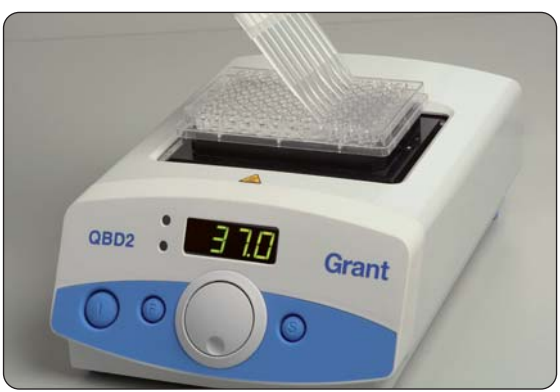


Figure 4: Aliquoting fresh Respiration Buffer (+/- pH-Xtra™)

STEP 3: Test compound stock or vehicle (typically 1-10µl) may be added at this point if desired. *NOTE: We recommend keeping the volume of added compound low to minimise any potential effects of solvent vehicle.*

STEP 4: Read the plate immediately in a fluorescence plate reader, with the set-up as described in Appendix A - Instrument Settings (Figure 5). The plate should be measured kinetically for >120 minutes. When the measurement is completed, remove the plate and save measured data to file.

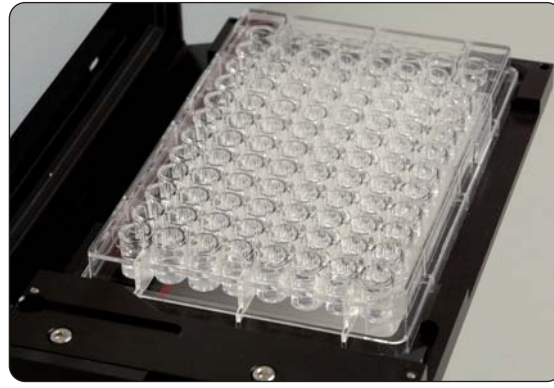


Figure 5: Reading the assay plate

Optional Controls

- **Signal Controls:** Leave 2 or 3 wells free from the addition of cells for use as Signal Control wells. Add 150µl of Respiration Buffer + 10µl of reconstituted pH-Xtra™ reagent to each well.

- **Positive Signal Controls:** Leave 2 or 3 wells free from the addition of cells for use as Positive Control wells. Add 150µl of fresh Respiration Buffer + 10µl of [1 mg/ml] glucose oxidase stock solution [in water] + 10µl reconstituted pH-Xtra™ reagent to each well.

- **Negative Controls:** To 2 or 3 wells containing cells, washed and refreshed with 150µl of Respiration Buffer, add 10µl of [750 mM] oxamic acid stock solution [in water] + 10µl reconstituted pH-Xtra™.

ANALYSIS

NOTE: We recommend that all first time users perform a Signal Optimisation test, as described. Signal and Blank Control wells may also be included.

ASSESSING EXTRACELLULAR ACIDIFICATION

Plot the Blank Control well-corrected pH-Xtra™ Intensity or Lifetime values versus Time (Figure 6). Select the linear portion of the signal profile (avoiding any initial lag or subsequent plateau) and apply linear regression to determine the slope (ECA) and correlation coefficient for each well. *NOTE: This approach is preferable to calculating a slope from averaged profiles.*

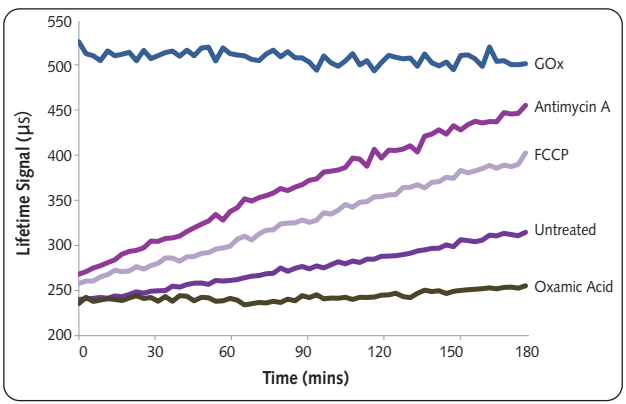


Figure 6: Typical Lifetime profile of pH-Xtra™ for adherant cells, treated with typical control compounds, including oxamic acid recommended as a negative control. The effect of glucose oxidase as a positive signal control is illustrated schematically. *NOTE: If using FCCP it is strongly recommended to perform a dose titration, since FCCP exhibits a bell-shaped dose response.*

Tabulate the slope values for each test sample, calculating appropriate average and standard deviation values across replicate wells. If optional Signal Control wells are included, the slope obtained for the Signal Control (sample without cells) should be subtracted from all test values.

Data analysis templates are available from some plate reader manufacturers, specifically configured to automate the analysis of Luxcel's pH-Xtra™ Glycolysis Assay. Microsoft Excel templates are also available through our website www.luxcel.com.

TITRATION OF CELL SEEDING DENSITY

To determine an optimal cell seeding density for performing the pH-Xtra™ Glycolysis Assay, for new cell types, seed replicate wells with a range of seeding densities (typically 0, 10,000, 20,000, 40,000, 60,000 and 80,000 cells/well). Plot the data generated as a function of intensity or Lifetime values versus time, as illustrated (Figure 7).

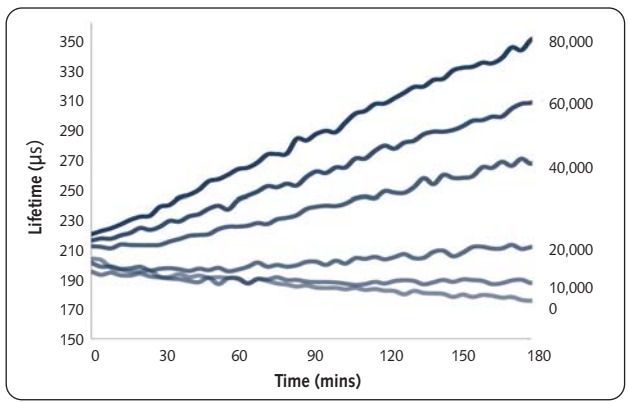


Figure 7: Extracellular Acidification rate profiles (ECA) are shown for A549 cells seeded at 0, 10,000, 20,000, 40,000, 60,000 and 80,000 cells/well. In this experimental example, a seeding density of 40,000 cells/well was chosen for study as this provided a suitable balance between ECA response and cell availability.

CELLULAR ENERGY FLUX ANALYSIS

Multiparametric (or multiplex) combination of pH-Xtra™ Glycolysis Assay together with Luxcel's MitoXpress® Xtra - Oxygen Consumption Assay [HS Method] (Cat No: MX-200) allows the simultaneous real-time measurement of glycolysis and mitochondrial respiration, and analysis of the metabolic phenotype of cells and the shift (flux) between the two pathways under pathological states (Figure 8).

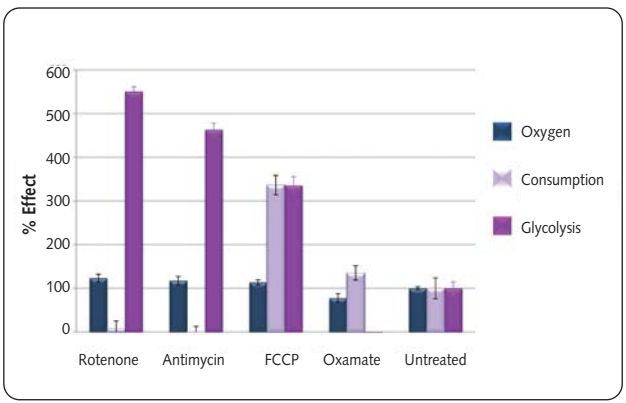


Figure 8: Cellular Energy Flux for HepG2 cells, treated with a combination of drug compounds modulating the ETC or inhibiting lactate production, shown as a percentage relative to untreated control cells. Comparative measurements with pH-Xtra™ (glycolysis) and MitoXpress® Xtra (oxygen consumption), show the shift between glycolysis and mitochondrial respiration and the cellular control of energy (ATP; measured 1h post-treatment using Promega Cell Titer-Glo®).

CALIBRATION OF pH-XTRA™ GLYCOLYSIS ASSAY TO A pH [H^+] SCALE

It is possible to express Extracellular Acidification (ECA) as a function of pH [H^+] versus time. This is achieved by first creating a calibration standard curve, by measuring TR-F intensity or preferably Lifetime values (selecting stabilised readings over a 30 minute read), from a range of pH-buffered standards at the appropriate assay temperature (see Figure 9). Select the linear portion of the standard curve and apply linear regression to determine the calibration function (See Hynes *et al.*, 2009).

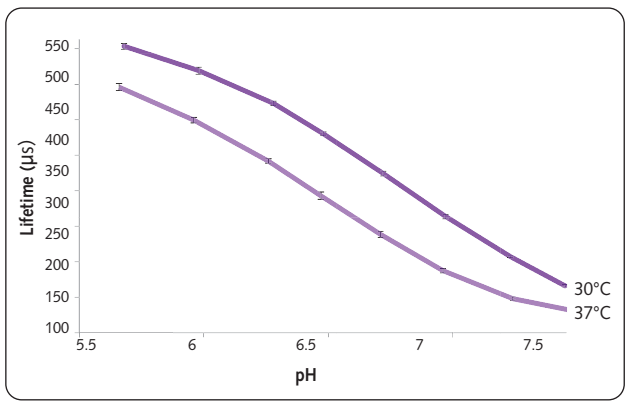


Figure 9: pH-XTRA™ reagent calibration in Lifetime scale, at 30°C and 37°C using pH-buffered PBS, at increments of 0.2 across a pH range 6.0 - 7.5.

APPENDIX A - INSTRUMENT SETTINGS

Two fluorescence modalities[1.] can be optimally used with the pH-Xtra™ Glycolysis Assay, depending on plate reader type and instrument setup. *NOTE: We strongly recommend only using fluorescence plate readers equipped with temperature control.*

Standard: TR-F Measurement
Measurement using time-resolved fluorescence (TR-F) provides flexibility to use a wide range of commonly available plate readers[2.]. TR-F measurement reduces non-specific background and increases probe sensitivity. Optimal delay time is ~100µs and gate (integration) time is 100µs. *NOTE: pH-Xtra™ should return a S:B ≥3.*

Advanced: Dual-Read TR-F (Lifetime)
Optimal performance can be achieved using dual-read TR-F in combination with subsequent ratiometric Lifetime calculation, to maximise dynamic range (Figure 10) and to express ECA as a function of $[H^+]$. *NOTE: pH-Xtra™ should return a S:B ≥10.*

Optimal dual-delay and gate (integration) times:
- Integration window 1: 100µs delay (D_1), 30µs measurement time (W_1)
- Integration window 2: 300µs delay (D_2), 30µs measurement time (W_2)

[1.] *pH-Xtra™ Glycolysis Assay may also be used in non - TR-F Intensity mode on some plate readers, although we recommend running the Signal Optimisation protocol to confirm an acceptable S:B, and optimising cells seeding density (Figure 7).*

[2.] *Users may see better performance using filter-based plate readers.*

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 pH-Xtra™ reagent, and can provide measurements of extracellular acidification that are more stable and with a wider dynamic range than measuring signal Intensity or standard TR-F. *NOTE: S:B for Integration window 2 is recommended to be ≥10 to allow accurate Lifetime calculation.*

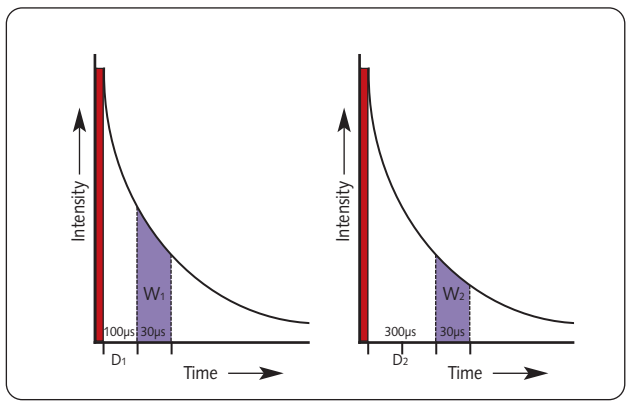


Figure 10: Illustrating dual-read TR-F measurement.

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

$$Lifetime\ (\mu s)[\tau] = (D_2 - D_1)/ln(W_1/W_2)$$

Where W_1 and W_2 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 ~200µs for cells assayed in respiration buffer at approx pH7.4, increasing up to >400µs upon acidification, and should only be calculated from samples containing pH-Xtra™ reagent. Lifetime values should not be calculated from blank wells.*

RECOMMENDED INSTRUMENT AND MEASUREMENT SETTINGS

Instrument	Optical Configuration	Integration 1 (D_1 / W_1) Integration 2 (D_2 / W_2)	Mode***	Ex (nm) Em (nm)
BMG Labtech:* FLUOstar Omega POLARstar Omega	Filter-based Top or bottom read	100 / 30µs 300 / 30µs	Dual-read TR-F (Lifetime)	Ex 340 ± 50nm (TR-EX L) Em 615 ± 10nm (BP-615)
BMG Labtech:* CLARIOstar**	Hybrid - filter based Top or bottom read	100 / 30µs 300 / 30µs	Dual-read TR-F (Lifetime)	Ex 340 ± 40nm Em 620 ± 10nm
BMG Labtech:* PHERAstar FS	Filter-based Top read (HTRF Module)	100 / 30µs 300 / 30µs	Dual-read TR-F (Lifetime)	Ex 337nm Em 620nm
BMG Labtech:* FLUOstar Optima / POLARstar Optima	Filter-based Top or bottom read	100 /100µs n/a	TR-F	Ex 340 ± 50nm (TR-EX L) Em 615 ± 10nm (BP-615)
Perkin Elmer: VICTOR series / X4, X5	Filter-based Top read	100 / 30µs 300 / 30µs	Dual read TR-F (Lifetime)	Ex 340 ± 40nm (D340) Em 615 ±8.5nm (D642)
Perkin Elmer: EnVision	Filter-based Top read	100 / 50µs 300 / 50µs	Dual-read TR-F (Lifetime)	Ex 340 ± 60nm (X340) Em 615 ±8.5nm (M615)
Perkin Elmer: EnSpire	Monochromator Top or bottom read	100 /100µs n/a	TR-F	Ex 380nm Em 615nm
BioTek:* Synergy H1, H4, HT, Neo, 2 Cytation 3**	Filter-based Top or bottom read	100 /30µs 300 / 30µs	Dual read TR-F (Lifetime)	Ex 360 ±40nm Em 620 ± 10nm
BioTek: MX, H1m	Monochromator Top or bottom read	100 /100µs n/a	TR-F	Ex 380nm Em 615nm
Tecan: Infinite / Safire / Genios Pro	Filter-based/Monochromator Top or bottom read	100 /100µs n/a	TR-F	Ex 380 ± 20nm Em 615 ± 10nm
Mol. Devices: SpectraMax / Flexstation / Gemini	Monochromator-based Top or bottom read	50 / 250µs n/a	TR-F	Ex 380nm Em 615nm

Notes: * Assay-specific protocols and notes are available from manufacturer for pH-Xtra™
** Assay-specific protocols in development (contact TechSupport@Luxcel.com)
*** Where TR-F indicated, a TR-F module must be installed

Note: 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 – TROUBLE SHOOTING

Extensive literature, including 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 pH-Xtra™ reagent should be stored between +2 to +8°C (see Use Before date on vial). *Reconstituted pH-Xtra™ reagent can be stored in the dark between +2 to +8°C for several days or stored as aliquots in water at -20°C for use within one month (avoid freeze thaw). Note: pH-Xtra™ reagent diluted in Respiration Buffer / media should be used on the same day.*

Respiration Buffer: Kit contains a single Respiration Buffer tablet sufficient for 50ml 1x stock, containing 1mM K-phosphate, 20mM Glucose, 70mM NaCl, 50mM KCl, 0.8mM $MgSO_4$, 2.4mM $CaCl_2$. Alternative media and supplements may be used as required (such as unbuffered DMEM), so long as care is taken to ensure a minimal buffering capacity.

Plate Reader: A fluorescence plate reader capable of measuring excitation between 360nm and 390nm (see Figure 2) and emission at 615nm, and having plate temperature control is required. We strongly recommend using TR-F measurement.

Temperature: We recommend the use of a plate block heater for plate preparation, 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, and inclusion of blank and optional additional control wells as described.

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 pH-Xtra™ reagent has been added, to reduce the potential for assay variability. Re-check pH of Respiration Buffer prior to use.

Cell Type and Cell Density: Since the pH-Xtra™ reagent measures Extracellular Acidification, the amount of signal change will be directly dependent on the rate of glycolytic flux of the cell type being measured. We recommend using a medium to high cell density per well as a starting point, and reducing cell numbers as required. (See Figure 7).

SIGNAL TO BLANK (S:B) OPTIMISATION

For most fluorescence plate readers, set up according to Appendix A - Instrument Settings, pH-Xtra™ should return a S:B ratio ≥3. The following options may be helpful to improve S:B if the ratio is not as high as expected:

1 Increase Gain (PMT) setting or flash energy
2 Adjust TR-F focal height
3 Increase length of integration time, the same for both delay windows.
4 Repeat as top or bottom-read, respectively.
5 Increase volume of pH-Xtra™ (15µl).
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 pH-Xtra™ (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. Check pH of pre-warmed Respiration Buffer and correct as necessary, as pH can drift over time. If tested and not resolved, contact TechSupport@luxcel.com

Q: What do I do if I can detect a signal in wells containing cells and pH-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 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.

REFERENCES

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Comparative bioenergetic assessment of transformed cells using a cell energy budget platform. Zhdanov AV *et al*, Integr. Biol., 2011; 3: 1135-1142

Fluorescent pH and oxygen probes of the assessment of mitochondrial toxicity in isolated mitochondria and whole cells. Hynes J *et al*, Curr Protoc Toxicol., 2009 May; Chapter 2: Unit 2.16

In vitro analysis of cell metabolism using a long-decay pH-sensitive lanthanide probe and extracellular acidification assay. Hynes J *et al*, Analytical Biochemistry., 2009; 390: 21-28

RELATED PRODUCTS

- MitoXpress® Xtra - Oxygen Consumption Assay [HS Method] (Cat No. MX-200)
- MitoXpress® Intra – Intracellular O_2 Assay (Cat No. MX-300)
- GreenLight® 960 – Microbial Detection Assay (Cat No. GL-960)

Luxcel Biosciences Limited

Suite 2.04

Western Gateway Building

Western Road

Cork

Ireland

t +353 (0)21 420 5348

e Sales@luxcel.com

w www.luxcel.com