CLARIOstar® *Plus* with ACU

Full flexibility for all your live cell-based assays

BMG LABTECH

The Microplate Reader Company

CLARIOstar® *Plus* with ACU

Hypoxic and ischemia/reperfusion conditions mimicked in a microplate reader

The closest to physiology

State-of-the-art cell culture employs biosensors to study biological changes arising from drugs or different genetic backgrounds in real-time. The aim is to conduct experiments that resemble physiological conditions as close as possible. This is achieved by addition of growth factors or by choosing particular culturing methods such as 3D cell culture. However, one major aspect is mostly neglected: oxygen (O_2) tension.

Within a human body, cells are exposed to O_2 concentrations that vary between 1 - 14 %, depending on the organ or tissue. In contrast, *in vitro* experiments are usually conducted at ambient O_2, i.e. approximately 20 %. These hyperoxic conditions affect cellular behaviour detrimentally. In fact, the O_2 environment impacts on gene and protein expression, alters the energy metabolism and secretion of soluble factors. The use of tissue-specific O_2 conditions will result in more reliable *in vitro* data that better translate to *in vivo* situations.

A model for ischemia/reperfusion

Disturbance of tissue-specific and stable O_2 conditions is often related to life-threatening diseases. Reduced O_2 and nutrient supply due to impaired blood flow occurs during stroke, myocardial infarction, shock, atherosclerosis and cancer.

Despite being essential for survival, reperfusion and sudden re-oxygenation of tissues upon ischemia induces significant cell damage through the formation of reactive oxygen species and activation of inflammatory responses. To date, *in vitro* solutions to study cellular reactions in response to ischemia and reperfusion are not amenable to real-time monitoring and are limited in throughput. Investigating these pathologies *in vitro* requires an experimental set-up capable of rapid deoxygenation and reperfusion.

The CLARIOstar® *Plus* with Atmospheric Control Unit (ACU) was engineered to not only provide stable atmospheric O_2 and CO_2 tensions, but also to run user-definable protocols of O_2 deprivation and reoxygenation.

For the first time you can fully manipulate the environment within the microplate reader, by mimicking *in vitro* hypoxia, ischemia/reperfusion and much more.

CLARIOstar *Plus* with Atmospheric Control Unit: full flexibility for all your live cell-based assays

Triple technology

The CLARIOstar *Plus* is a multi-mode, high-performance plate reader with a revolutionary new type of monochromator technology. Along with the advanced LVF Monochromators™, this modular and upgradable reader is equipped with filters and a UV/vis spectrometer, that can be used for a variety of applications in the following detection modes:

- UV/vis absorbance
- Fluorescence intensity, including FRET
- Fluorescence polarization/anisotropy
- Time-Resolved Fluorescence, including TR-FRET
- Luminescence (flash & glow), including BRET
- AlphaScreen®/AlphaLISA®/AlphaPlex™

LVF Monochromator™ technology

LVF Monochromators are based on Linear Variable Filters which consists of two aligned slides that separate light into distinct wavelengths and continuously adjustable bandwidths. The CLARIOstar contains two LVF Monochromators, one for excitation and one for emission. Since LVF Monochromators separate light differently than conventional monochromators, they provide significantly higher sensitivity.

Simplify your assay setup

The CLARIOstar *Plus* makes detection optimization simpler than it has ever been. With Enhanced Dynamic Range (EDR) and rapid, full-plate autofocus, every sample on your plate is automatically measured with the best possible settings. EDR grants a dynamic range spanning over 8 concentration decades in a single measurement, providing an easier solution for assay development and cell-based kinetic analysis.

The rapid, full-plate auto-focus, gives excellent sensitivity in all plate formats up to 1536 wells. Combined with EDR, it makes detection easier and improves data quality.

Perfect environment

The Atmospheric Control Unit (ACU) module can regulate CO_2 and O_2, reproducing within the reader physiological as well as hypoxic conditions needed for live cell-based assays. In combination with temperature control, three different shaking options, bottom reading detection, and Z-height focus adjustment, the ACU provides an ideal 'walk-away' solution for any long-term cell-based assay.

The ACU features include:

- O_2 and CO_2 control range: 0.1-20 %
- O_2 and CO_2 gas ramps
- Gas value trackability in MARS data analysis software
- Intuitive interface with touchscreen, up to 10 gas pre-sets and gas concentration curve display
- Altitude correction for accurate gas regulation

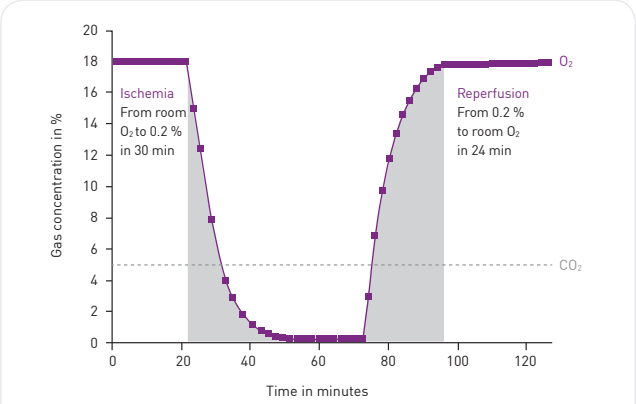

Fig. 1: *Example of ischemia/reperfusion conditions mimicked in the CLARIOstar *Plus* with ACU.*

Gas ramping

As a unique feature within plate readers, the CLARIOstar *Plus* offers the capability to run O_2 and CO_2 gas ramps. For instance, the ACU can deprive O_2 and then rapidly re-oxygenate back to physiological conditions, keeping meanwhile steady CO_2 levels. This capability enhances live cell-based assays, as disease models such as ischemia/reperfusion can be reproduced *in vitro* in a microplate reader.

Applications include:
Proliferation - Cell viability - Bacterial growth - Migration and invasion - Hypoxia - Angiogenesis - Ischemia/reperfusion - Cytotoxicity studies - Viral uptake - Intracellular pH

The CLARIOstar *Plus* with ACU exposes cells to ischemia/ reperfusion conditions and monitors their oxygenation

C. Carey, J. Hynes (Luxcel Biosciences Ltd., Cork, Ireland), M. Schwalfenberg, R. Kettenhofen (Axiogenesis AG, Cologne, Germany)

Introduction

The lack of oxygen supply is associated with a number of life-threatening diseases whereby cells are temporarily deprived of O_2 and nutrient (ischemia). Significant cell damage can also occur during the reperfusion phase through oxidative stress and inflammation. Investigating these pathologies *in vitro* requires an experimental set-up capable of rapid deoxygenation, rapid reperfusion, and parallel monitoring of critical biological parameters including cellular oxygenation and Reactive Oxygen Species (ROS).

Assay principle

The ischemia/reperfusion model presented here uses the CLARIOstar *Plus* plate reader with programmable O_2 and CO_2 regulation in combination with MitoXpress®-Intra, (Luxcel Biosciences) which enables real-time monitoring of cellular oxygenation. Data are presented using HepG2 cells and iPS derived cardiomyocytes (Cor.4U®, Axiogenesis). Mitochondrial membrane potential (MMP) was determined with the JC-1 fluorescent indicator. Induction of ROS was reported by the redox-sensitive fluorophore dihydroethidium (DHE).

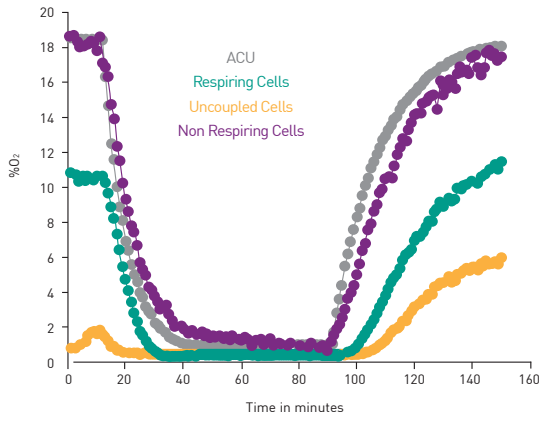

Fig. 2: *Ischemia/reperfusion proof-of-concept using HepG2 cells. Ischemia/reperfusion insult induced by modulating O_2 in the measurement chamber. Cellular oxygenation is monitored in respiring, non-respiring (Antimycin treated), and uncoupled (FCCP treated) cells.*

Results & discussion

The CLARIOstar *Plus* microplate reader equipped with software-controlled programmable O_2 and CO_2 regulation achieves precise atmospheric control, with O_2 reduced to 1 %, maintained low for 50 min and then rapidly increased to 18 % (fig.2). Real-time monitoring of oxygenation reveals the impact of cellular respiration and ambient O_2 on O_2 concentrations at the cell monolayer. Antimycin treated HepG2 cells (no respiration), reflect instrument conditions (ACU). However, respiring cells experience much lower resting O_2 concentrations and deeper more sustained hypoxia. This disparity between atmospheric and cellular O_2 increases further when respiration is increased through FCCP treatment (uncoupled cells).

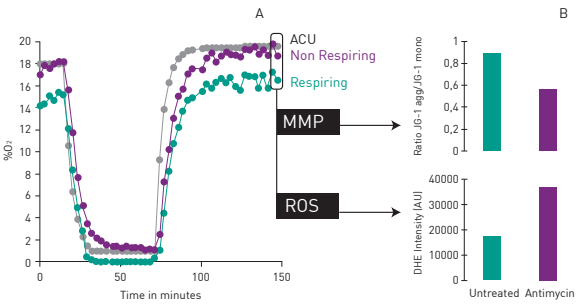

Fig. 3: *Multiparametric analysis of Cor4U cells during in vitro ischemia/ reperfusion validating multiplexed measurement of MitoXpress-Intra and JC-1/DHE. Cell oxygenation traces describe depth and duration of Cor.4U ischemia-reperfusion **(A)** with parallel monitoring of MMP and ROS **(B)**.*

The approach was also evaluated using iPS-derived cardiomyocytes (Cor.4U) with parallel monitoring of MMP and ROS using the CLARIOstar *Plus*' convenient multiplexing function. Respiring cells experience significantly reduced O_2 concentrations while antimycin treated cells reflected ACU conditions (fig. 3A) and also causing MMP dissipation as well as increased ROS production (fig. 3B).

Conclusion

- O_2 ramping of ACU facilitates control of ischemic and reperfusion insults in cells
- Intracellular probe tracks cellular oxygenation during ischemia/reperfusion cycle
- Parallel monitoring of ROS and MMP probes allow detailed metabolic characterization of ischemia-reperfusion

Mitochondrial oxidant generation follows oxygen deprivation and re-oxygenation

Daniel Pastor-Flores and Tobias Dick, German Cancer Research Center (DKFZ), Heidelberg, Germany

Introduction

Yeast is a popular eukaryotic model organism because it is easy to genetically modify and robust to differing environments. Plated on agar plates, it can be studied under aerobic conditions. However, studying transition of agar-plated yeast to anaerobic conditions requires atmospheric control to reduce O_2 tension. The Singer Instruments ROTOR device pins colonies of yeast onto agar plates in 96, 384 or 1536 plate format and enables high-throughput studies. The CLARIOstar^{Plus} plate reader with ACU exposes yeast colonies to a desired O_2 and CO_2 atmosphere and detects their fluorescence.

Assay principle

The dependence of oxidant formation on ambient O_2 was measured in yeast clones expressing mito-roGFP2-Tsa2DCR, a fluorescent mitochondrial redox-sensitive sensor. In addition, yeast cells expressed a citrate synthase 2 (Cit2) fusion-protein with mCherry. Cit2 is upregulated upon activation of the retrograde pathway, a common marker of mitochondrial dysfunction. Auto-fluorescence of yeast NAD(P)H corrected for the growth of yeast colonies. Yeast was pinned onto agar plates resembling the layout of a 384 well plate. Changes in O_2 pressure were achieved by the ACU.

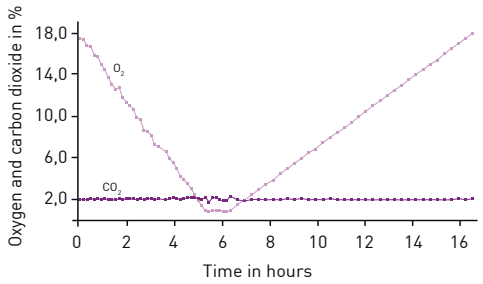

Fig. 4: *Oxygen and carbon dioxide levels in the course of the experiment.*

Results & discussion

Yeast was exposed to varying levels of O_2 (fig. 4) and the redox state of mitochondrial roGFP2 was investigated. During the period of O_2 decrease (0 –1.2 h) from 18 % to 12 %, the redox state of mitochondrial H_2O_2 probe was not influenced by the decreasing O_2. This points to a favoured O_2 supply to mitochondria over the cytosol in case of fluctuating O_2 concentrations. At lower O_2 saturation, the amount of the reduced probe increased as reported by lower roGFP2 ratios. While the O_2 is kept at 1 % (5-6.5 h), the probe persists in its reduced form and gets oxidized only in the phase of re-oxygenation. The retrograde pathway is induced upon inhibition of mitochondrial respiration and reports mitochondrial failure to the nucleus. A protein synthesized as a result of pathway activation is Cit2. During O_2 deprivation, Cit2 expression is slightly reduced whereas it remarkably increases during reperfusion (fig. 5). Whether the Cit2 increase is due to reperfusion or is a delayed response to hypoxic conditions, remains to be elucidated.

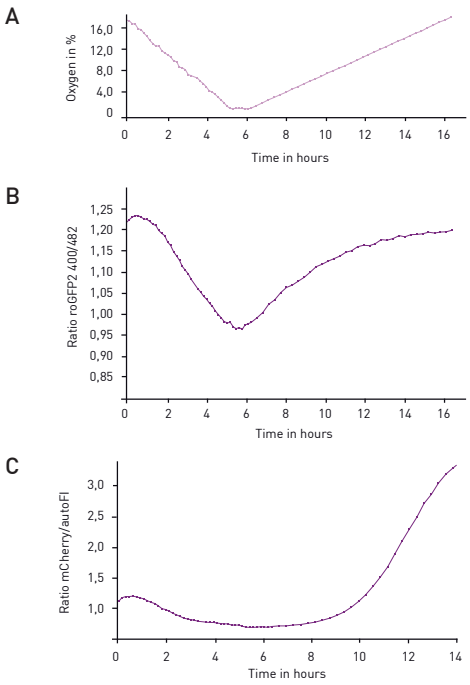

Fig. 5: *The influence of O_2 availability (A) on the redox state of mito-chondrial peroxiredoxin-based probe (B) and Cit2 expression (C). O_2 pressure modulates the redox state of a genomic-integrated Mito-roGFP2-Tsa2ΔCR probe. roGFP2 oxidation is represented by 400 nm/480 nm ratio and indicative of H_2O_2 generation (B). Cit2 expression is reported by CIT2-mCherry fusion protein corrected for growth by NAD(P)H autofluorescence (C).*

Conclusion

- Measurement of fluorescent yeast colonies on agar
- Investigation of normoxic and hypoxic conditions using the CLARIOstar^{Plus} with atmospheric control unit
- Measurement of multiple parameters employing the flexibility of the LVF Monochromators™

CLARIOstar *Plus* - Technical specifications

The CLARIOstar *Plus* can include all or any combination of features listed below at purchase. Upgrading with additional features is possible at any time. Please contact your local representative for more details or a quote.

Detection modes	UV/vis absorbance spectra Fluorescence intensity (incl. FRET) Luminescence (flash and glow) - incl. BRET Fluorescence polarization Time-resolved fluorescence TR-FRET AlphaScreen®/AlphaLISA®/AlphaPlex™	
Measurement modes	Top and bottom reading Endpoint and kinetic Sequential multi-excitation Sequential multi-emission Spectral scanning (fluorescence, luminescence, absorbance) Ratiometric measurements Well scanning	
Microplate formats	6- to 1536-well plates, user-definable LVis Plate with 16 low volume microspots (2 µL)	
Microplate carrier	Robot compatible	
Light sources	High energy xenon flash lamp Dedicated laser for AlphaScreen®/AlphaLISA®/AlphaPlex™	
Detectors	Low-noise photomultiplier tube Red-sensitive photomultiplier tube CCD spectrometer	
Wavelength selection	Dual Linear Variable Filter (LVF) Monochromators™ Linear Variable Dichroic Mirror: separates excitation and emission LVF Monochromators Optical filters: excitation and emission slides hold up to 4 filters each LVF Monochromators + optical filters: use one for excitation and the other for emission UV/vis absorbance spectrometer: full spectra or 8 discrete wavelengths in < 1 sec/well	
Optical filters	Excitation and emission slides for up to 4 filters each	
Optical path	Top and bottom: free-air optical light path guided by motor-driven mirrors and dichroics	
Z-Adjustment	Automatic focal height adjustment (0.1 mm resolution)	
Spectral range	**Filters**	FI, FP, TRF: 240 - 740 nm or 240 - 900 nm (red-shifted PMT) LUM: 240 - 740 nm
	LVF Monochromators™	FI: 320 - 740 nm or 320 - 840 nm (red-shifted PMT) LUM: 320 - 740 nm
	Linear Variable Dichroic	340 - 740 nm or 340 - 760 nm (red-shifted PMT)
	Spectrometer	ABS: 220 - 1000 nm
Sensitivity	**FI filters** (top)	< 0.15 pM (< 3 amol/well fluorescein, 384sv, 20 µL)
	FI filters (bottom)	< 1.0 pM (< 50 amol/well fluorescein, 384g, 50 µL)
	FI monochromator (top)	< 0.35 pM (< 7 amol/well fluorescein, 384sv, 20 µL)
	FI monochromator (bottom)	< 3.0 pM (< 150 amol/well fluorescein, 384g, 50 µL)
	FI dynamic range	8 decades in a single measurement
	FP	< 0.5 mP SD at 1 nM fluorescein (384sv, 20 µL)
	HTRF® (black and white microplates)	Reader Control Kit (Eu) after 18h (384sv, 20 µL) Delta F > 880 % (High Calibrator) Delta F > 30 % (Low Calibrator)
	TRF	< 20 fM europium, 384, 80 µL
	LUM	< 0.4 pM (< 8 amol/well ATP, 384sv, 20 µL)
	LUM dynamic range	8 decades in a single measurement
	AlphaScreen® with laser	< 5 pM (< 100 amol/well P-Tyr-100, 384sv, 20 µL)
	ABS with spectrometer	Full spectrum captured in < 1 s / well Selectable spectral resolution: 1, 2, 5, and 10 nm OD range: 0 - 4 OD Accuracy: < 1% at 2 OD Precision: < 0.5% at 1 OD and < 0.8% at 2 OD
Read times	**Flying mode** (1 flash)	8 s (96), 15 s (384), 28 s (1536)
	10 flashes	19 s (96), 57 s (384), 3 min 4 s (1536)
Reagent injection	Up to 2 built-in reagent injectors Individual injection volumes for each well: 3 to 500 µL (optionally up to 2 mL) Variable injection speed up to 420 µL/s Reagent back flushing	
Shaking	Linear, orbital, and double-orbital with user-definable time and speed	
Integrated barcode reader	Up to two integrated barcode readers	
Incubation	+3°C above ambient up to 45°C or 65°C The upper heating plate of the incubation chamber operates at 0.5 °C more than the lower plate. This prevents condensation build-up on the lid or sealer.	
Software	Multi-user Reader Control and MARS data analysis software included **FDA 21 CFR Part 11 compliant** Integrated fluorophore library	
Dimensions	Width: 45 cm, depth: 51 cm, height: 40 cm; weight: 32 kg	
	Optional accessories	
Stacker	Plate handler for up to 50 microplates - continuous loading feature	
THERMOstar	Microplate incubator and shaker	
Atmospheric Control Unit (ACU)	Actively regulates O_2 and CO_2 - 0.1-20% Gas ramping function	
LVis Plate	Microplate designed to measure 16 low volume (2 µL) samples and standard cuvettes. Incorporating NIST-traceable filters and holmium oxide standards for instrument performance test. Sensitivity: < 2 ng/µL dsDNA	
Filters	Optimized for dyes, fluorophores and specific assays Filters for all applications from UV to NIR Customized filters available upon request	
Upgrades	Upgrades to include options such as additional detection modes, reagent injectors, extended temperature control, etc. are available. Please contact your local representative for more information.	

US Patent Number 9,733,124. US Patent Number 6,700,690 used under license.
Limit of detection (sensitivity) was calculated according to the IUPAC standard: 3x(SD_{blank})/slope
Specifications are subject to change without notice.

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