CLARIOstar® with ACU

Full flexibility for all your live cell-based assays

BMG LABTECH

The Microplate Reader Company

Hypoxic and ischaemia/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 ischaemia/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 ischaemia 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 ischaemia 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® 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, ischaemia/reperfusion and much more.

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

Triple technology

The CLARIOstar 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 for several reasons:

- **Avoidance of stray light** as it occurs in conventional monochromators decreases background signal and significantly increases sensitivity
- Unique **continuously adjustable bandwidths** from 8 to 100 nm allow more light for excitation and emission
- A **Linear Variable Dichroic** mirror reduces background by separating excitation and emission light
- The inline optical system can **combine filter and monochromator** within the same measurement, e.g. exciting with a filter and scanning the emission
- **Spectral scanning** is possible for top and bottom in both fluorescence and luminescence modes

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:

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

- 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

Gas ramping

As a unique feature within plate readers, the CLARIOstar 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 ischaemia/reperfusion can be reproduced *in vitro* in a microplate reader.

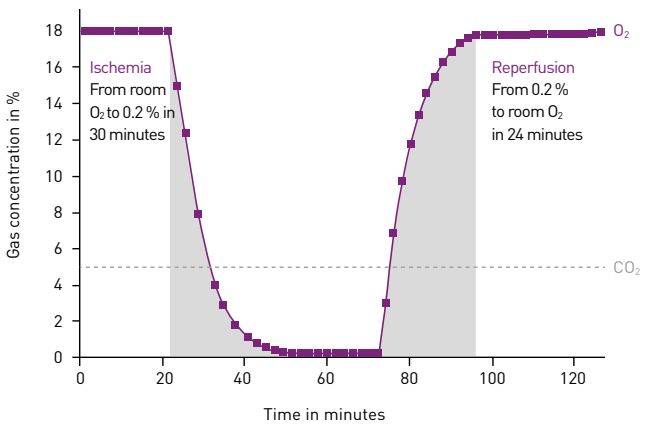

Fig. 1: *Example of ischaemia/reperfusion conditions mimicked in the CLARIOstar with ACU.*

The CLARIOstar with ACU exposes cells to ischaemia/ 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 (ischaemia). 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 ischaemia/reperfusion model presented here uses the CLARIOstar 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).

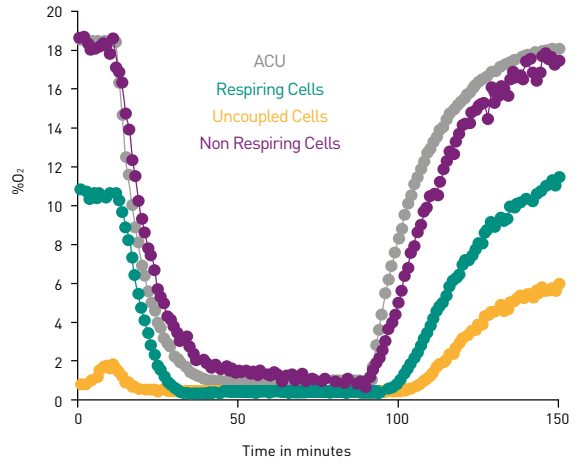

Fig. 2: *Ischaemia/reperfusion proof-of-concept using HepG2 cells. Ischaemia/reperfusion insult induced by modulating O_2 in the measurement chamber. Cellular oxygenation is monitored in respiring, non-respiring (Antimycin treated), and uncoupled (FCCP treated) cells.*

Results & discussion

The CLARIOstar 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).

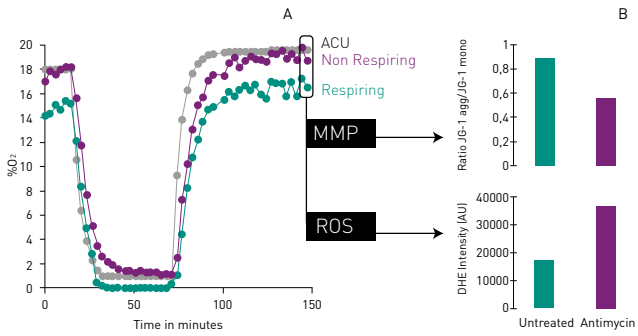

Fig. 3: *Multiparametric analysis of Cor4U cells during in vitro ischemia/ reperfusion validating multiplexed measurement of MitoXpress-Intra and JC-1/DHE. Cell oxygenation traces describe depth and duration of Cor.4U ischemia-reperfusion **(A)** with parallel monitoring of MMP and ROS **(B)**.*

The approach was also evaluated using iPS-derived cardiomyocytes (Cor.4U) with parallel monitoring of MMP and ROS using the CLARIOstar's 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 ischaemia/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 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.

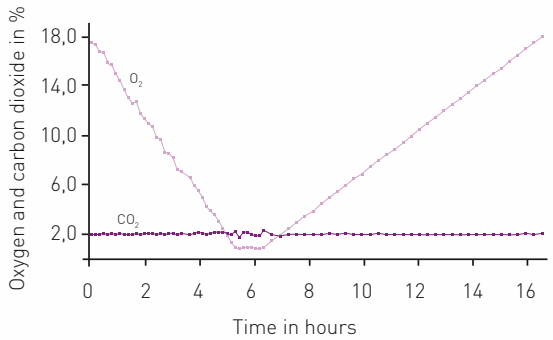

Fig. 4: *Oxygen and carbon dioxide levels in the course of the experiment.*

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.

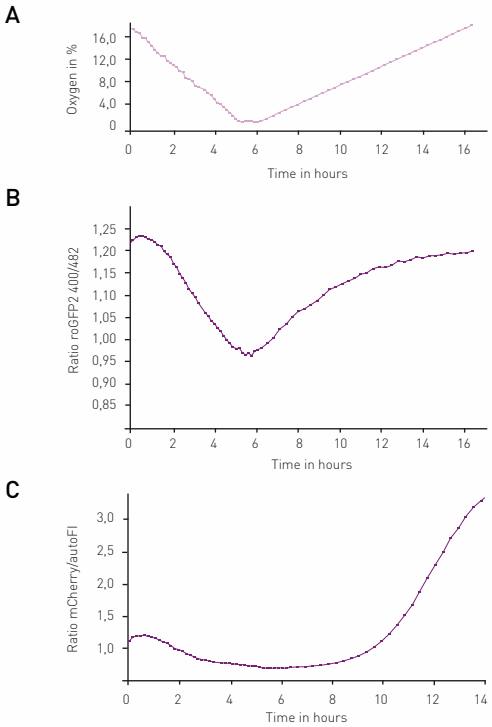

Fig. 5: *The influence of O_2 availability (A) on the redox state of mitochondrial peroxiredoxin-based probe (B) and Cit2 expression (C) . O_2 pressure modulates the redox state of a genomic-integrated Mito- roGFP2-Tsa2ΔCR probe. roGFP2 oxidation is represented by 400nm/480nm ratio and indicative of 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 with atmospheric control unit
- Measurement of multiple parameters employing the flexibility of the LVF-monochromators™

CLARIOstar® - Technical specifications

Due to the modularity of BMG LABTECH's instruments, all, or combinations of the features below can be installed at purchase or upgraded at any time. Please contact your local representative for more details or a quote.

Detection modes	Fluorescence intensity - including FRET Luminescence (flash and glow) - including BRET UV/vis absorbance Fluorescence polarization / anisotropy Time-Resolved Fluorescence - including TR-FRET AlphaScreen® / AlphaLISA® / AlphaPlex™	
Measurement modes	Top and bottom reading Endpoint and kinetic measurements Spectral scanning (fluorescence, luminescence and absorbance) Well scanning	
Microplate formats	6- to 1536-well plates, LVis Plate with 16 microspots (2 µL)	
Light sources	High energy xenon flash lamp Dedicated laser for AlphaScreen® / AlphaLISA®/AlphaPlex™	
Detectors	Low noise Photomultiplier Tube (PMT) UV/vis absorbance spectrometer	
Dual LVF Monochromator™	Fluorescence, luminescence: top and bottom Fluorescence excitation / emission spectral scanning Luminescence emission spectral scanning Spectral range: 320 - 850 nm (selectable increments from 0.1 to 10 nm) Software selectable bandwidths: 8 - 100 nm	
Linear Variable Dichroic Mirror	Spectral range: 340 - 740 nm (selectable increments of 0.1 nm)	
UV/vis absorbance spectrometer	Spectral scanning or up to 8 discrete wavelengths in less than 1 sec / well Spectral range: 220 - 1000 nm (selectable increments from 1 to 10 nm) Bandwidth: 3 nm	
Optical filters	Top and bottom for all detection modes, except absorbance Up to 4 excitation filters, 4 emission filters, and 3 dichroic mirrors Spectral range: 240 - 900 nm	
Sensitivity*	FI LVF Monochromator	Top: < 0.35 pM fluorescein, 384sv, 20 µL (< 7 amol/well) Bottom: < 3.0 pM fluorescein, 384, 50 µL (< 150 amol/well)
	FI Filters	Top: < 0.15 pM fluorescein, 384sv, 20 µL (< 3 amol/well) Bottom: < 1.0 pM fluorescein, 384, 50 µL (< 50 amol/well)
	FP	< 0.5 mP SD at 1 nM fluorescein, 384sv, 20 µL
	TRF	< 20 fM europium, 384, 80 µL
	HTRF®	HTRF® certified for black and white microplates Reader Control Kit (Eu) after 18h incubation, 384sv, 20 µL > 880 % Delta F for High Calibrator > 30 % Delta F for Low Calibrator < 2.0 % CV for Standard 0
	LUM	< 0.4 pM ATP, 384sv, 20 µL (< 8 amol/well) Dynamic Range: 9 decades
	AlphaScreen®	< 100 amol/well P-Tyr100 (384sv, 20 µL)
	ABS with spectrometer	Accuracy: < 1% at 2 OD Precision: < 0.5% at 1 OD and < 0.8% at 2 OD Dynamic range: 0 - 4 OD
Read times	1 flash: 8 s (96) 15 s (384) 28 s (1536) 10 flashes: 19s (96) 57 s (384) 184 s (1536)	
Reagent injection	Up to 2 built-in reagent injectors with reagent back flushing Individual injection volumes for each well 3 to 500 µL (optional up to 2 mL) Variable injection speed up to 420 µL/s	
Shaking	Linear, orbital, and double-orbital with user-definable time and speed	
Incubation	+3°C above ambient to 45°C (65°C optional)	
Software	Integrated fluorophore library Multi-user software package including Reader Control and MARS data analysis software, FDA 21 CFR Part 11 compliant	
Dimensions	Width: 45 cm, depth: 51 cm, height: 40 cm; weight: 32 kg	
Accessories		
ACU	Actively regulates O_2 and CO_2: 0.1 - 20%	
LVis Plate	Measure 16 low-volume samples (2 µL) and QC standards	
Stacker	Magazines for up to 50 plates - continuous loading feature	

BMG LABTECH's LVF Monochromator includes technology covered under US Patent 6,700,690, for which BMG LABTECH has an exclusive license for the microplate reader market.
*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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