Mitochondrial oxidant generation follows oxygen deprivation.

Device pins colonies of yeast onto agar plates in 96, 384 or 1536 concentrations. At lower O2 saturation, the amount of redox sensitive probe increased as reported by lower roGFP2 fluorescence.

Results & discussion

Plated yeast to anaerobic conditions requires atmospheric CO2 environments. Plated on agar plates, it can be studied under atmospheric O2 deprivation. Cit2 is upregulated upon inhibition of mitochondrial respiration and response to hypoxic conditions, remains to be elucidated. The influence of O2 availability on the redox state of mitochondrial B and A is shown in the figure.

Investigation of normoxic and hypoxic conditions using the CLARIOstar® Plus with ACU.

Full flexibility for all your live cell-based assays.

Measurement of fluorescent yeast colonies on agar plates under varying O2 concentrations. Yeast was exposed to varying levels of O2 (Fig. 4)

Yeast is a popular eukaryotic model organism because of their fluorescence. NAD(P)H corrected for the growth of yeast colonies. Yeast fusion-protein with mCherry. Cit2 is upregulated upon addition, yeast cells expressed a citrate synthase 2 (Cit2) fluorescent mitochondrial redox-sensitive sensor. In the figure, the Cit2 increase is due to reperfusion or is a delayed response. Whether it remarkably increases during reperfusion (Fig. 5).

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conditions mimicked in Hypoxic and CLARIOstar® soluble factors. The use of tissue-specific O2 concentrations that vary between 1 - 14 %, depending on the organ or tissue. In contrast, in vitro experiments are usually conducted at ambient O2, i.e. approximately 20 %. These hyperoxic conditions affect cellular behaviour detrimentally. In fact, the O2 environment impacts on gene and protein expression, alters the energy metabolism and secretion of soluble factors. The use of tissue-specific O2 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 O2 conditions is often related to life-threatening diseases. Reduced O2 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 reoxygenation. The CLARIOstar® Plus with Atmospheric Control Unit (ACU) was engineered to not only provide stable atmospheric O2 and CO2 tensions, but also to run user-definable protocols of O2 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 CO₂ and O₂, 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:
- O₂ and CO₂ control range: 0.1-20 %
- O₂ and CO₂ 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® Plus offers the capability to run O₂ and CO₂ gas ramps. For instance, the ACU can deprive O₂ and then rapidly re-oxygenate back to physiological conditions, keeping meanwhile steady CO₂ 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 O2 and nutrient (ischemic). 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 O2 and CO2 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).

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 O2 on O2 concentrations at the cell monolayer. Antimycin treated HepG2 cells (no respiration), reflect instrument conditions (ACU). However, respiring cells experience much lower resting O2 concentrations and deeper more sustained hypoxia. This disparity between atmospheric and cellular O2 increases further when respiration is increased through FCCP treatment (uncoupled cells).

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 O2 concentrations while antimycin treated cells reflected ACU conditions (fig. 3A) and also causing MMP dissipation as well as increased ROS production (fig. 3B).

Conclusion
- O2 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

Results & discussion
The CLARIOstar Plus microplate reader equipped with software-controlled programmable O2 and CO2 regulation achieves precise atmospheric control, with O2 reduced to

![Fig. 2: Ischemia/reperfusion proof-of-concept using HepG2 cells. Ischemia/reperfusion insult induced by modulating O2 in the measurement chamber. Cellular oxygenation is monitored in respiring, non-respiring (Antimycin treated), and uncoupled (FCCP treated) cells.](image-url)
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 O₂ 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 O₂ and CO₂ atmosphere and detects their fluorescence.

Assay principle
The dependence of oxidant formation on ambient O₂ 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 O₂ pressure were achieved by the ACU.

Results & discussion
Yeast was exposed to varying levels of O₂ (fig. 4) and the redox state of mitochondrial roGFP2 was investigated. During the period of O₂ decrease (0 – 1.2 h) from 18 % to 12 %, the redox state of mitochondrial H₂O₂ probe was not influenced by the decreasing O₂. This points to a favored O₂ supply to mitochondria over the cytosol in case of fluctuating O₂ concentrations. At lower O₂ saturation, the amount of the reduced probe increased as reported by lower roGFP2 ratios. While the O₂ 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 O₂ 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.

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™
Mitochondrial oxidant generation follows oxygen deprivation

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

Yeast was exposed to varying levels of O2 (Fig. 4) and the redox environments. Plated on agar plates, it can be studied under well-scanning conditions. It is easy to genetically modify and robust to differing oxygen tensions. Control to reduce O2 tension. The Singer Instruments ROTOR CLARIOstar® Plus - Technical specifications

Detection modes

UVs 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)

Light sources

High-energy xenon flash lamp, Dedicated laser for AlphaScreen®/AlphaLISA®, AlphaPlex™

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, UVVis 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


Sensitivity

FI filters (top): < 0.15 pm (× 3 amoL/well fluorescein, 38ks, 20 µL), FI filters (bottom): < 1.0 pm (× 50 amoL/well fluorescein, 38ks, 50 µL), FI monochromator (top): < 0.35 pm (× 7 amoL/well fluorescein, 38ks, 20 µL), FI monochromator (bottom): < 3.0 pm (× 15 amoL/well fluorescein, 38ks, 50 µL), FI dynamic range: 8 decades in a single measurement

FP: < 0.5 mV/SD at 1 nm fluorescein (38ks, 28 µL), HTRF® (black and white microplates): Reader Control Kit [Eu] after 18 h [38ks, 20 µL], Delta F: > 800 % [High Calibrator], Delta F: > 30 % [Low Calibrator], TRF: ≥ 20 FM europium, 38ks, 80 µL, LUM: ≥ 0.4 pm (× 8 amoL/well ATP, 38ks, 20 µL), LUM dynamic range: 8 decades in a single measurement, AlphaScreen® with laser: < 5 µM (× 100 amoL/well P-Tyr-100, 38ks, 20 µL), 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 (Wt), 15 s (38ks), 28 s (1536), 10 flashes: 19 s (Wt), 57 s (38ks), 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

+2°C above ambient up to 45°C or 40°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 MAPS 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

Optical accessories

Open reagent injectors, reagent injection, extended temperature control, etc. are available. Please contact your local representative for more information.

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.

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.

Specifications are subject to change without notice.

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