

Cell viability

Apoptosis

Necrosis

ROS production

Flexible solutions for your cell-based assays

Migration

Gene expression

Signaling pathways

Proliferation



Dedicated solutions from cell viability to pharmacological profiling



A "walk-away" platform

Cell-based assays are used in life science research and in drug discovery. Their constant gain in popularity is due to the fact that they better reflect the complexity of biological systems when compared to biochemical approaches.

BMG LABTECH plate readers provide a versatile platform that can cover a multitude of cellular assays. With gas and temperature control, they provide a true "walk-away" solution that eliminates the need to shuffle plates to and from the incubator, increasing throughput and reducing manual intervention.

Viability, proliferation, and toxicity

Viability assays are based either on metabolic activity measured with colorimetric assays (e.g., MTT), or on ATP production, most frequently measured by luminescence. As viable cells do not necessarily proliferate, specific assays like the use of labelled nucleosides or nuclear stains have been developed to differentiate between the two.

Cytotoxicity assays report on cell death-specific events such as loss of membrane integrity with membrane-impermeable dyes, caspase activation with colorimetric, fluorescent or luminescent readouts, or changes on the cell surface like phosphatidylserine increase measured by binding to Annexin V with luminescence. All these endpoint or real-time kinetic assays can be effortlessly measured in a microplate reader.

Migration assays

Cell migration analysis includes wound healing and transwell assays. Migration is most easily monitored with cells expressing fluorescent or luminescent tags or by the use of cell dyes. All these assays can be measured efficiently and in real-time over long kinetics in a plate reader.

Expression assays

Fluorescence or luminescence readouts are typically used as proxy for the introduction of new genetic material in cells or for expression analyses. Reporter gene assays, transfection efficiency and viral infection with modified fluorescent viruses can all be monitored in real-time for days.

Cell signaling and interaction assays

Signaling cascades can be measured in different ways including transcriptional activation, phosphorylation by kinases or engagement of secondary messengers. These assays typically measure an analyte as either an endpoint or kinetic assay and rely on a direct light output or on signal production using fluorescence, luminescence, FRET, TR-FRET or AlphaScreen®. BRET assays are specifically suited for the analysis of protein interactions in real-time and can also be used for pharmacological profiling in living cells.

Key features improve sample handling and data quality

Temperature incubation

The optimal temperature for most eukaryotic cells is 37°C. At this temperature cells provide physiological responses and display optimal growth rates.

BMG LABTECH readers offer precise temperature control at any desired temperature up to 45°C. The incubation chamber consists of two independent heating plates, above and below the microplate. The upper plate operates at 0.5°C higher than the lower plate to prevent condensation and provide uniform incubation.

Gas regulation

To better understand cellular mechanisms, many assays rely on the analysis of live cells in a real time kinetic fashion. To keep responses unbiased, cells have to be exposed to physiological levels of O₂ and especially CO₂. The Atmospheric Control Unit (ACU) module independently regulates the O₂ and CO₂ concentrations within the plate chamber, producing optimal culture conditions. Cellular samples can be kept at stable gas and temperature conditions for days, while kinetic data can be automatically acquired in real-time.



The Atmospheric Control Unit perfectly regulates both O₂ and CO₂.

Gas ramping

As a unique feature, the CLARIOstar Plus with ACU offers the capability to run O₂ gas ramps. For instance, the ACU can deprive O₂ down to 0.1% and then rapidly re-oxygenate back to physiological conditions, while keeping CO₂ levels steady. With this capability, disease models such as ischemia/reperfusion or cancer-driven hypoxic cycles can be mimicked *in vitro* in a microplate reader.

Bottom reading

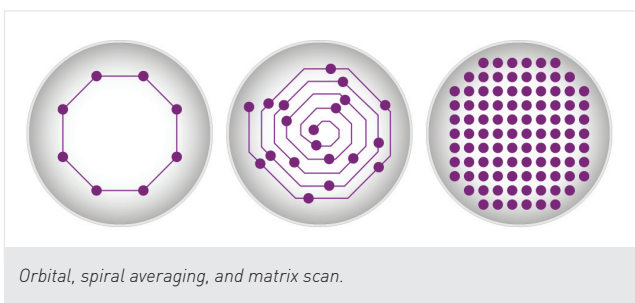
When analysing adherent cells, detection from the bottom of the microplate significantly improves data quality. On our microplate readers, you can easily switch from top to bottom detection

with a mouse click - no manual hardware adjustments are required. Bottom reading can be applied to any detection mode, both with LVF Monochromators™ or using filter detection.

Well scan modes for heterogeneous samples

As adherent cell layers are most often non-homogenous, measuring only in the centre of the well may deliver inaccurate results. Signal detection over the well surface is usually beneficial, as it delivers more robust data with less variability.

On our readers, three different well scan modes enable accurate data acquisition even from non-homogeneous samples. Orbital and spiral averaging automatically normalise for heterogeneous or non-confluent cell distribution. For higher resolution, matrix scan can acquire up to 900 data points/well, displaying each scan point graphically and creating a map for each well. Single scan points or entire sections can easily be excluded after detection.



Orbital, spiral averaging, and matrix scan.

Flexible reagent dispensing

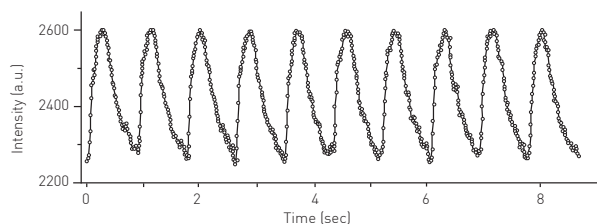
Reagent dispensers can be used to add a stimulus or inhibitor to samples to allow for rapid signal changes seen as a result to be captured. With our injectors, delivery volume, timing, and speed are independently adjustable for each sample for plate formats up to 384-well. An extremely low dead volume and back-flush capability ensure precious reagents are used sparingly and can be recovered.

On the VANTASTAR, injectors can be combined with a software-controlled heater and magnetic stirrer, enabling reagent mix and dispensing from a bottle or beaker at a specific temperature.

High-frequency sampling

Cellular responses are often very quick and can rapidly change. The CLARIOstar Plus and PHERASTAR FSX have a sampling rate of up to 100 measurements/second. High-frequency sampling acquires extremely fast changing signals and kinetics like calcium flux or fast biological reactions with ease.

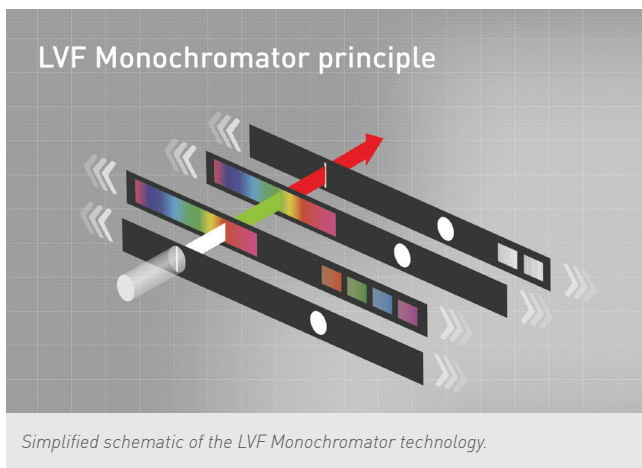
Combined with the capability of simultaneously dispensing reagents and quantifying samples, it ensures no data point will be lost.



Detection of Ca^{2+} transient measurements using heart tissue and Fluo-4.

Wavelength flexibility

The capability to freely select the excitation and emission wavelengths depending on the required assay significantly enhances flexibility. Our patented LVF Monochromators™ ensure wavelength flexibility combined with filter-like performance, and the spectral scanning capability required for assay development. A UV/vis spectrometer enables full absorbance spectral acquisition (220 - 1000 nm) in less than 1 second/well.



Multiplexing capability

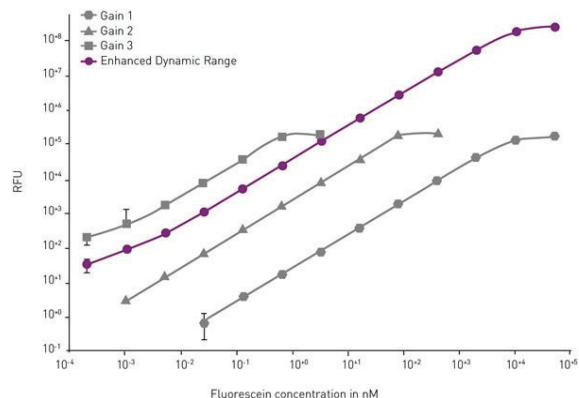
Multi-parametric analysis increases the yield of information obtained from one single cell sample and provides a more holistic approach. For multiple readout analyses, fluorescent detection of up to 5 different dyes can be combined with absorbance and luminescence in the same run. This allows for the parallel characterisation of different parameters in cells or the normalisation of reporter assays.

Simplified assay setup

In cell-based kinetic assays, it is often difficult to find the optimal gain settings as signal intensities typically increase over time.

The Enhanced Dynamic Range (EDR) technology grants a dynamic range spanning over 8 concentration decades in a single measurement. EDR significantly simplifies detection setup as it ensures a reliable detection of highly divergent signals, with no manual intervention.

EDR helps to avoid detector saturation in kinetic assays and guarantees that every sample is automatically measured with the ideal settings.



EDR enables the detection of samples spanning over 8 concentration decades in one single measurement with no manual intervention.

Red dye sensitivity

The use of red fluorophores is usually beneficial when detecting fluorescent readouts in cells, as most cellular components produce auto-fluorescence in the blue-green range.

Users who need the very best performance in far-red fluorescent detection can benefit from a red-sensitive PMT on the CLARIOstar Plus. The use of an additional dedicated detector for luminescence and AlphaScreen provides the option to measure with the most sensitive PMT for your detection mode without compromise.

Fast detection of dual emission assays

Interaction assays such as ligand-receptor binding often require the detection of two emission wavelengths. Simultaneous Dual Emission (SDE) detection enables the concomitant acquisition of two separate emission signals in one single measurement. In FRET, TR-FRET, BRET, FP, and AlphaPlex™, SDE halves read times, reduces data variability, allowing for a more dynamic measurement of interactions to be achieved.

Comprehensive data analysis

The multi-user MARS software is designed to make data analysis and data export simple and effective. It provides an extensive range of data processing tools and is fully compliant with FDA regulation 21 CFR Part 11.

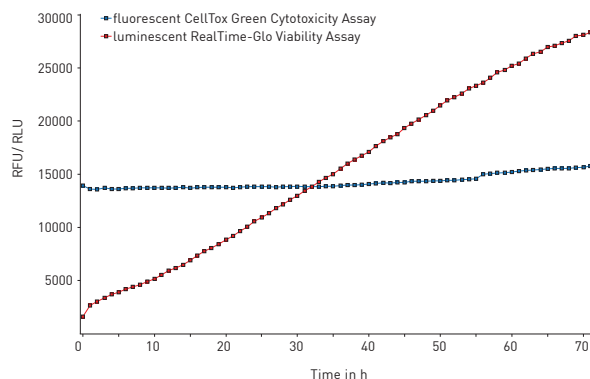
Analysis of cell viability and cytotoxicity in a 72-hour long kinetic

Real-time cell kinetic assay with combined fluorescence and luminescence readouts

Real-time detection of cell viability and cytotoxicity allows identification of the exact moment in time when a cytotoxic or anti-proliferative event occurs. On a microplate reader the two assays can be multiplexed, enabling the detection of cell viability and cytotoxicity in parallel in the same sample.

K562 cells in 384-well microplates were treated with a panel of test compounds (e.g., tyrosine kinase inhibitor bosutinib) with known effects on proliferation and cytotoxicity. Promega's RealTime-Glo[®] MT Cell Viability and the CellTox[™] Green Cytotoxicity Assay were measured on a CLARIOstar equipped with Atmospheric Control Unit (ACU). The plate reader was used to both incubate the cells and to quantify luminescence and fluorescent signal changes every hour for 72 hours.

Results of untreated cells show that even after a 72-hour incubation in the reader cells were viable and not affected by cytotoxicity.



Multiplexed RealTime-Glo and CellTox[™] Green assay. Average results of 10 replicates shows that the amount of viable cells is increasing while cytotoxicity is unchanged over 72 hours in untreated cells.

For more information please scan the QR code or refer to BMG LABTECH application note 278.



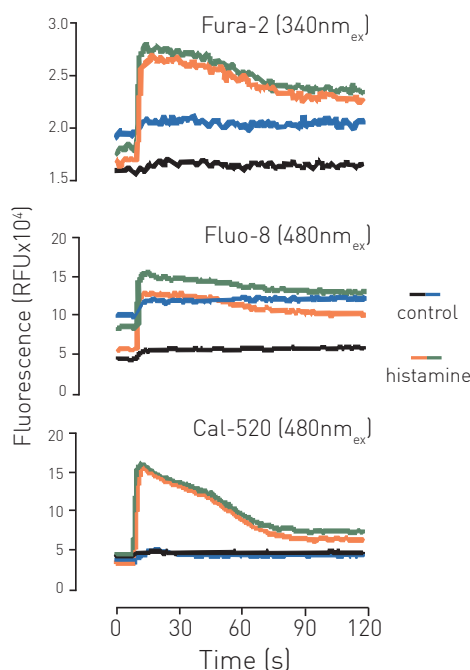
Real-time monitoring of intracellular Ca²⁺ concentration

Ca²⁺ transients were measured in real-time with high temporal resolution in endothelial cells

Changes in the intracellular concentration of Ca²⁺ ions are the basis for numerous cellular responses. Fluorescent imaging has long been used to monitor intracellular Ca²⁺ levels in living cells. Our microplate readers have the ability to control gas and temperature, read from the bottom of the well with a high sampling rate, and inject reagents automatically. They have allowed the adaptation of this fluorescence-based assay to a microplate format, greatly increasing its throughput.

This application note compares 3 commercially available fluorescent Ca²⁺ dyes (Fura-2AM, Fluo-8AM and Cal-520AM) used to monitor histamine-stimulated Ca²⁺ mobilisation in HUVEC cells in a 96-well plate. Ca²⁺ release upon stimulation was measured on a CLARIOstar with ACU set at 37°C and 5% CO₂.

Cal-520AM and Fura-2 accurately reported Ca²⁺ mobilisation with minimal leakage over a 30-minute time frame, making these dyes useful for mid/high-throughput analysis in living cells.



Intracellular Ca²⁺ measurements in a 96-well plate using various calcium dyes. Cells were loaded with 2µmol/L dye and stimulated with either ddH₂O (control) or histamine (10µmol/L). Duplicate traces are shown.

For more information please scan the QR code or refer to BMG LABTECH application note 333.



Transfection efficiency of HeLa cells expressing GFP and mcherry

Reliable determination of cell transfection efficiency on a microplate reader

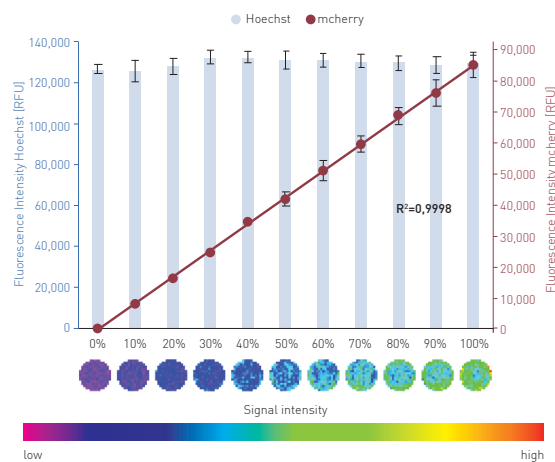
Transfection efficiency in cells is typically monitored with a microscope, using a fluorescent reporter gene as proxy for the expression of a gene of interest.

The percentage of transfected cells can also be efficiently evaluated using a microplate reader. To mimic transfection efficiency, HeLa cells stably expressing GFP and mcherry were mixed in increasing ratios with wild-type HeLas without fluorescent reporter.

Fluorescence signals of GFP, mcherry, and Hoechst 33342 (for normalisation) were quantified on the VANTastar either with matrix scan or spiral averaging, using bottom detection.

Results confirm a linear relationship between the percentage of fluorescent cells and the GFP or mcherry signals measured on the plate reader with high accuracy and precision.

The VANTastar was able to reliably detect the fraction of GFP- or mcherry- expressing cells down to 5.3% and 3.1%, respectively.



Linear relationship of percentage of GFP+/mcherry+ HeLas (= transfection efficiency) and obtained mcherry signal with matrix scan. Error bars refer to 8 replicates. Matrix scan example shown for one well each.

For more information please scan the QR code or refer to BMG LABTECH application note 367.

Mimicking and monitoring of ischemia-reperfusion conditions *in vitro*

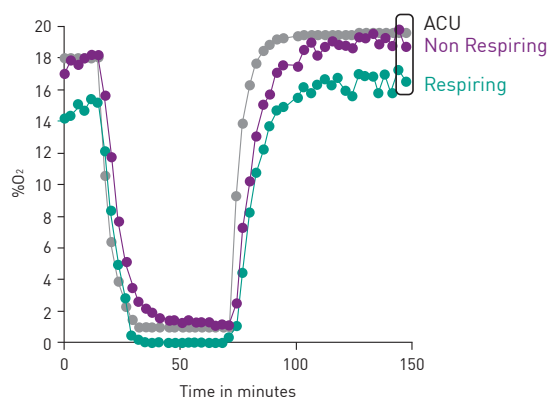
The CLARIOstar^{Plus} exposes cells to ischemia-reperfusion while analysing cell metabolism

The lack of oxygen supply is associated with a number of life-threatening diseases such as stroke or myocardial infarction. However, significant cell damage occurs as well during the reperfusion phase through oxidative stress and inflammatory responses.

Investigating ischemia-reperfusion *in vitro* requires an experimental set-up capable of rapid deoxygenation, rapid reperfusion, and parallel monitoring of critical biological parameters including cellular oxygenation and ROS production.

The CLARIOstar^{Plus} microplate reader with ACU enables the simulation of a hypoxic insult of defined depth and duration, and rapid controlled reperfusion. The reader was used to induce a defined ischemia-reperfusion event *in vitro* in liver cells and in iPS-derived cardiomyocytes.

A multi-parametric analysis of key cellular indicators such as real-time oxygenation, ROS production, and mitochondrial membrane potential was performed in the plate reader during the ischemia-reperfusion event.



Ischemia-reperfusion proof-of-concept using iPS-derived cardiomyocytes. Cellular oxygenation is monitored in respiring, non-respiring (Antimycin treated), and uncoupled (FCCP treated) and ACU is shown to indicate the O₂ levels in the reader.

For more information please scan the QR code or refer to BMG LABTECH application note 309.

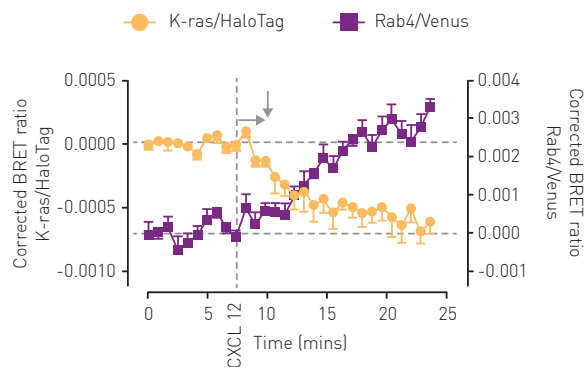
Multiplex GPCR interaction and trafficking analysis with NanoBRET™

Interaction assays with endogenous receptor levels enabled by CRISPR/Cas9 and NanoBRET™

GPCRs are important drug targets and require receptor-protein interaction and trafficking studies to reveal how they function. Although NanoBRET is a versatile tool to study interactions, it is limited by the ectopic expression of labelled interaction partners. CRISPR/Cas9 editing overcomes this limitation by enabling endogenous expression of luciferase-labelled proteins.

To study GPCR internalization and trafficking, Nluc was fused to endogenously-expressed CXCR4. Cells were additionally transiently co-transfected with K-ras-HaloTag and Rab4-Venus fusion proteins serving as plasma membrane and early endosome marker, respectively.

This multiplex assay was rendered possible as Venus and HaloTag signals could be successfully separated using the LVF Monochromator. Respective BRET ratios were determined upon CXCL12 treatment.



Cells expressing genome-edited CXCR4 fused to Nluc and transiently co-transfected with HaloTag/K-ras and Rab4/Venus were used to study genome-edited CXCR4/Nluc receptor internalization and trafficking induced by CXCL12 in the same cell.

For more information please scan the QR code or refer to BMG LABTECH application note 316.



Real-time cell motility tracking of wound healing assays

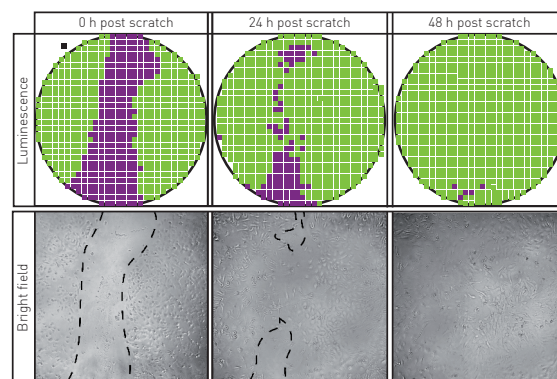
Scratch assay with auto-bioluminescent cells performed on a plate reader with well scanning

Scratch wound assays are useful for studying healing responses, cell interactions and migration. While the common scratch assay is reasonably simple, its dependence on microscopy makes it low throughput.

Here, we show a scratch assay performed with auto-bioluminescent cells in 96-well plates. The assay was detected in real-time, using the well-scanning detection capabilities of the CLARIOstar Plus with ACU.

Well scan luminescent readings on the plate reader paralleled the microscope images, showing the utility of this method for the evaluation of a wound assay. Moreover, the results of the quantitative analysis on the reader correlated with the visual results of the scratch wound assay.

Plate readers can assist in generating quantitative results with increased throughput. Acquired data can be used to compare treatments and to determine their effect on wound healing.



Comparison of bright-field micrographs on a microscope with microplate reader detection of a scratch wound assay with well scanning and a 30x30 matrix.

For more information please scan the QR code or refer to BMG LABTECH application note 372.



