INCYTON

CYRIS flox

Metabolism and
morphology at a glance

CYRIS® FLOX

– physiometabolic real-time cell analysis

INCYTON®'s incubator-integrated cell analysis platform monitors and analyzes several key cellular parameters in real-time and label-free. CYRIS® simultaneously measures:

- Cell viability
- Metabolism
- Morphology

These are captured in CYRIS® by oxygen consumption rate, extracellular acidification rate, cellular impedance and imaging. The measurements are performed in a fully automated, freely adjustable atmosphere-controlled environment.

PERFORM THE FOLLOWING EXPERIMENTS WITH CYRIS®

TOXICOLOGY
Standard toxicological substance analysis

METABOLIC RESEARCH
Mitochondrial profiling with Mito-Stress Test

BASIC CELLULAR RESEARCH
On/Off studies with complex addition and removal of substances

ONCOLOGY/HYPOXIA RESEARCH
Mimic physiological oxygen conditions of tumor microenvironments

Our principal research areas are toxicology, drug screening, metabolic research, basic cellular research, oncology, hypoxia research.

WHAT'S MAKING CYRIS® THE GO-TO CELL ANALYSER?

Freely programmable sequences of experiments and output of the raw and processed data

Unlimited addition and removal of substances and nutrients by an automated media supply system

Simultaneous measurement of four parameters (OCR, ECAR, impedance and live microscopic cell images)

CYRIS ACE –
The intuitive software with touch screen enabled controls and embedded data analysis functions

An innovative plate design and the interaction with a pipetting robot enable assays with adherent and suspension cells for short- and long-term experiments with well-specific fractionated supernatant collection

Full incubator integrated control of temperature, atmospheric oxygen and humidity

Parallel investigation and treatment of 24 independent cell samples using a microplate format

Rapid and sensitive label-free optical and electronic microsensor detection in real time

High-precision automated inverse microscope with phase-contrast like imaging in high resolution and an ultra-fast mechanical autofocus

High contrast microscopy lighting for cell-type specific imaging using light patterns provided by the 48 LED array

THE APPLICATIONS

INCYTON® technologies are applicable to various fields, including toxicological assessments, oncology, metabolic profiling, and drug screening, as well as cellular research in general. Our assays help to understand complex cellular relationships through the simultaneous observation of several key cell parameters. In combination with real-time monitoring, the kinetics of substance responses can be identified.

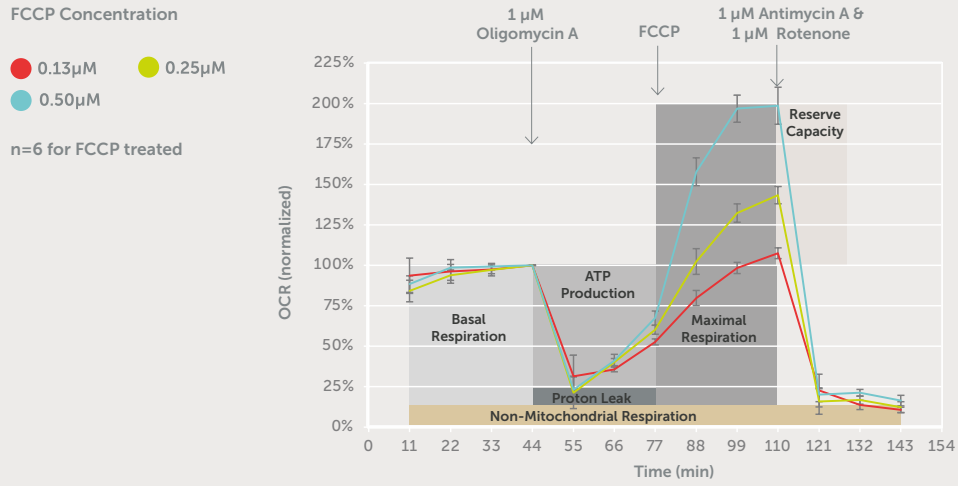
MITOCHONDRIAL STRESS TEST

This test is utilized to create a kinetic profile of key parameters of mitochondrial functions by measuring the OCR of cells under the influence of different inhibitors and uncouplers within 6-8 h (including cell seeding). Thereby, the basal respiration, ATP production, proton leakage, maximal respiration, reserve capacity, and non-mitochondrial respiration can be defined.

Below is shown the procedure of the CYRIS® analysis platform, with adherent cells of the cell line HepG2 as an example. In addition, continuous microscopic images, as seen below, can be used to monitor the process.

The results show that all OCRs measured were evaluable, consistent and all values have only a small standard deviation. By using this assay, you can gain insight into the usable oxygen levels for ATP production and define the maximum respiration of your cell line, besides obtaining valuable information on basal respiration, reserve capacity and non-mitochondrial oxygen consumption at the same time.

Because of very local effects on the respiratory chain, continuous imaging here serves more to control the process than to record changes.



Temporal course of the OCR while HepG2 cells undergo a standard Short Mitochondrial Stress Test.

All key parameters of mitochondrial function can be evaluated.

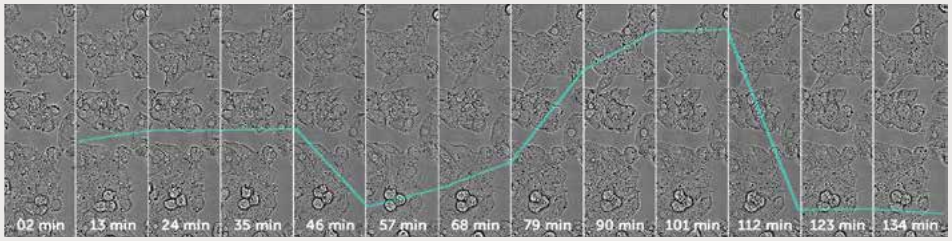
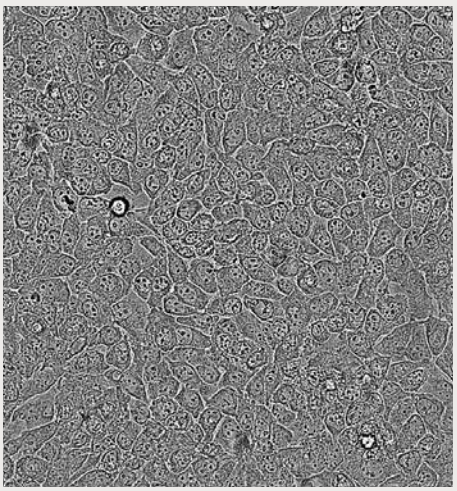


Image series of microscopic magnifications and corresponding OCR-course of HepG2 cells treated with 0.5 µM FCCP. When looking at the cells treated with the highest concentration of FCCP (0.5 µM), a consistently vital picture with only minor movements of the cells can be seen. For a better overview, we have overlaid the course of the oxygen consumption of these cells on the image series.

HIGH-RESOLUTION INVERTED MICROSCOPE

Our live-cell imaging technology allows real time and long-term sharp visualization of cell behavior giving you greater insights into toxicity and drug testing.



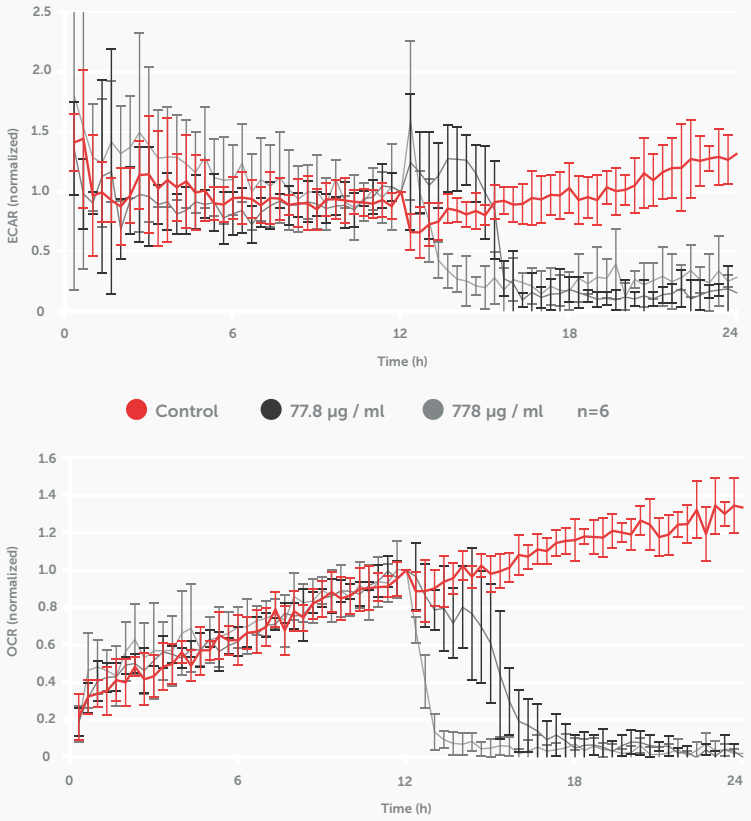
L929 mouse fibroblasts after short treatment with 0.5 µM FCCP. The cells show normal viability.

TOXICITY ASSAY

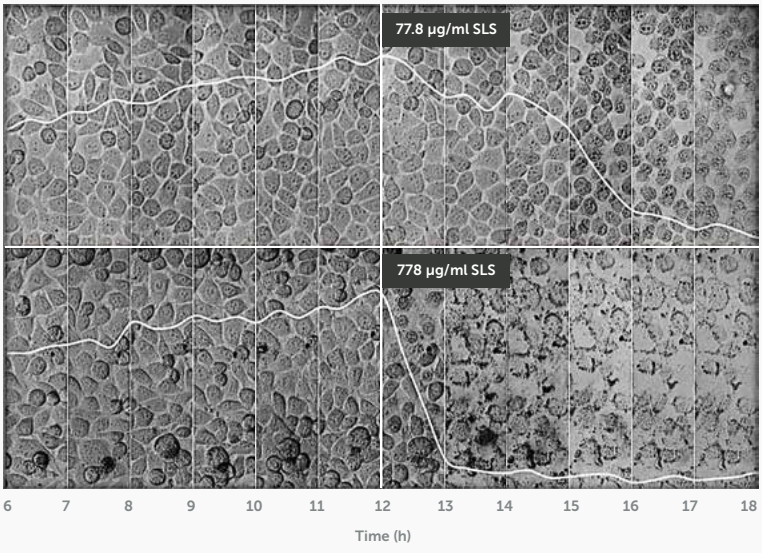
This assay demonstrates the power of a combined real-time sensor system and a microscope. L929 mouse fibroblast cells were investigated in the CYRIS® analysis platform under the influence of the reference toxicant sodium lauryl sulfate (SLS). The test settings included 12 h of pretreatment and 12 h of treatment with different concentrations of SLS. Throughout the assay, OCR and ECAR, as well as morphology based on regular microscope images, were recorded.

The results of the metabolic monitoring clearly show the concentration-dependent change in cellular OCR and ECAR in a quantitative and temporal profile. The medium SLS concentration (77.8 µg/ml) demonstrates how multiple sensing devices can identify the temporal mechanisms of the action of a substance. In the first 3 h after SLS addition, the OCR is reduced, while ECAR increases.

Responsible for these opposite effects are rapidly occurring damages to the mitochondrial membranes and the attempt of the cell to compensate for this by increased glycolysis. After continuation of the treatment, the damage to all the cell membranes finally leads to cell death and a drop in all the metabolic values.



OCR and ECAR of L929 cells under treatment with different concentrations of SLS (0 µg/ml, 77.8 µg/ml, 778 µg/ml) show a clear dose- and time-dependent reaction. After the initial impairment of the respiratory chain, at first the cells try to compensate for this loss by higher glycolytic activity (ECAR).



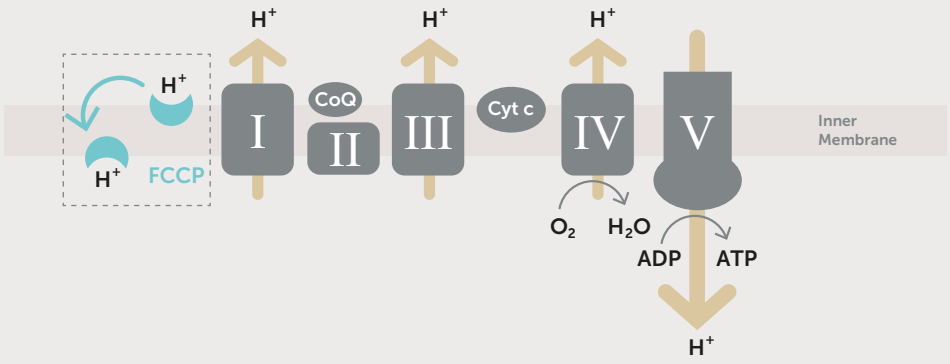
CORRELATION OF SENSOR DATA

Because the exact time points of every measuring and imaging event are known, it is possible to correlate these data for comparison. Clear correlations are visible by comparing the OCR with the hourly recorded microscopic images of the cells.

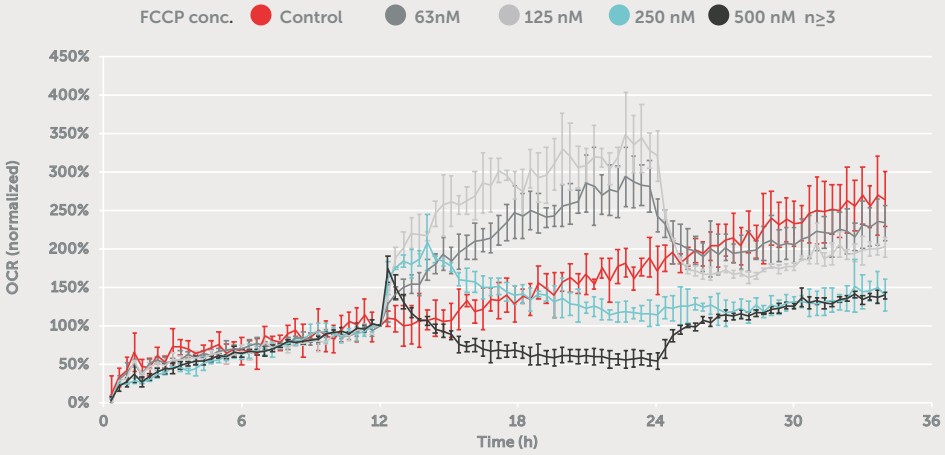
UNCOUPLER WASHOUT ASSAY

This in-vitro assay can be used to evaluate the effects of drugs that directly affect the mitochondrial respiratory chain. Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) is a potent uncoupler of oxidative phosphorylation in mitochondria and was used as an example to show the effects on mitochondrial function.

The testing of L929 mouse fibroblasts treated with FCCP in the CYRIS® analysis platform demonstrates a strong effect of FCCP on mitochondrial integrity and proves that this effect is reversible by washout of FCCP. This is enabled by the monitoring of metabolic parameters such as OCR and ECAR but also by continuous microscopic imaging over the course of the entire experiment.



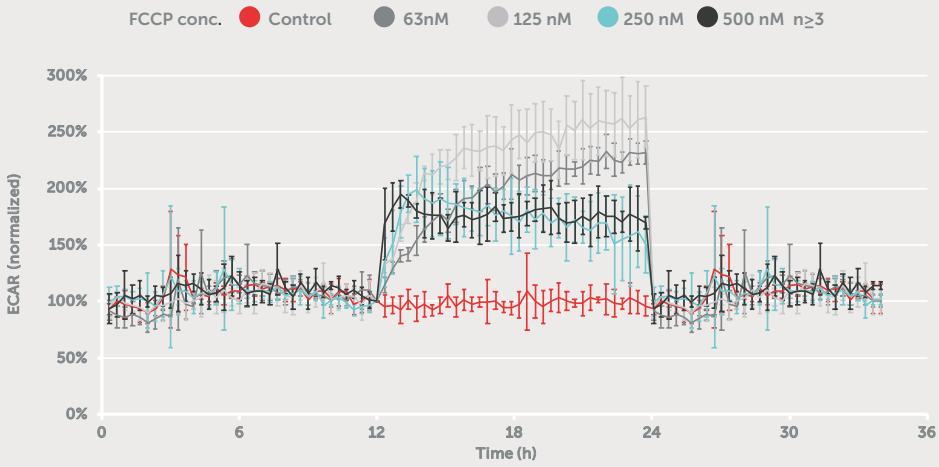
The site of action of FCCP in the mitochondrial respiratory chain.



OCR of L929 cells under the influence of different FCCP concentrations.

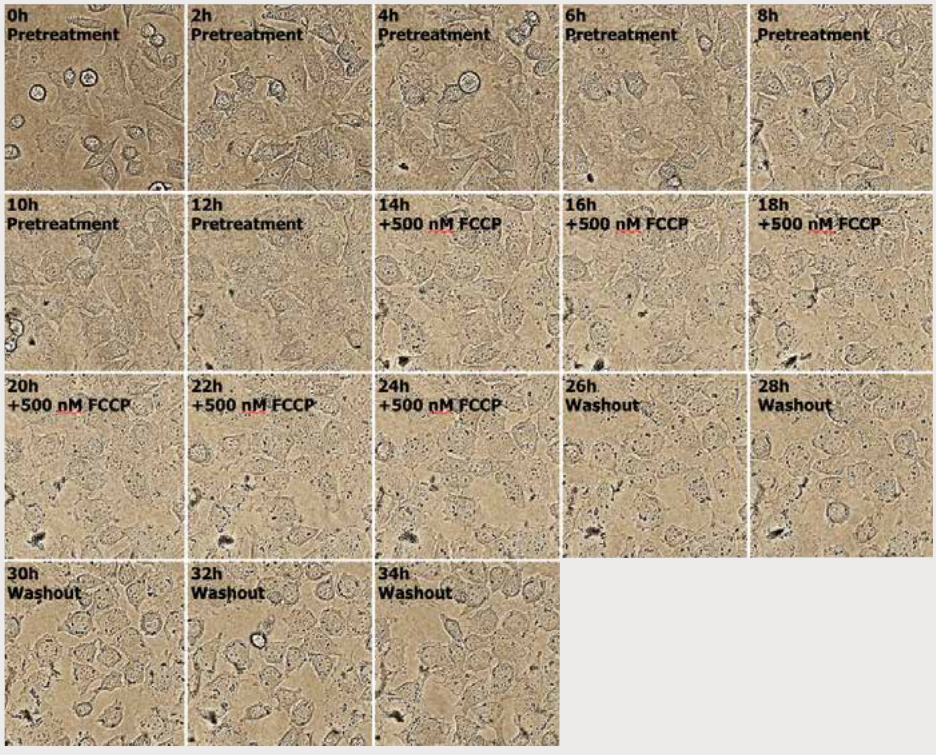
The influence of FCCP on L929 fibroblasts shows a clear time- and dose-dependent effect on OCR and ECAR, as illustrated below. The OCR of the control group grows steadily.

At lower FCCP concentrations (63 nM and 125 nM), the cells compensate for the membrane uncoupling effect of FCCP through increased oxygen-driven proton transport, which is reflected by an increase in the OCR of the cells. The higher turnover in the respiratory chain and proliferation is also reflected in the ECAR, which increases steadily at lower FCCP concentrations.



ECAR of L929 cells under the influence of different FCCP concentrations.

At higher FCCP concentrations (250 nM and 500 nM), the uncoupling effect of FCCP is so strong that the respiratory chain can no longer operate. The cells strongly decrease their consumption of oxygen and their proliferation, as indicated by a decrease in the OCR of the cells. In addition, the ATP production via the respiratory chain is reduced. To compensate for this, the cells produce more ATP via glycolysis, which is characterized by a persistently increased acidification.



Magnified imaging of L929 fibroblasts treated with 500 nM FCCP and washout of FCCP.

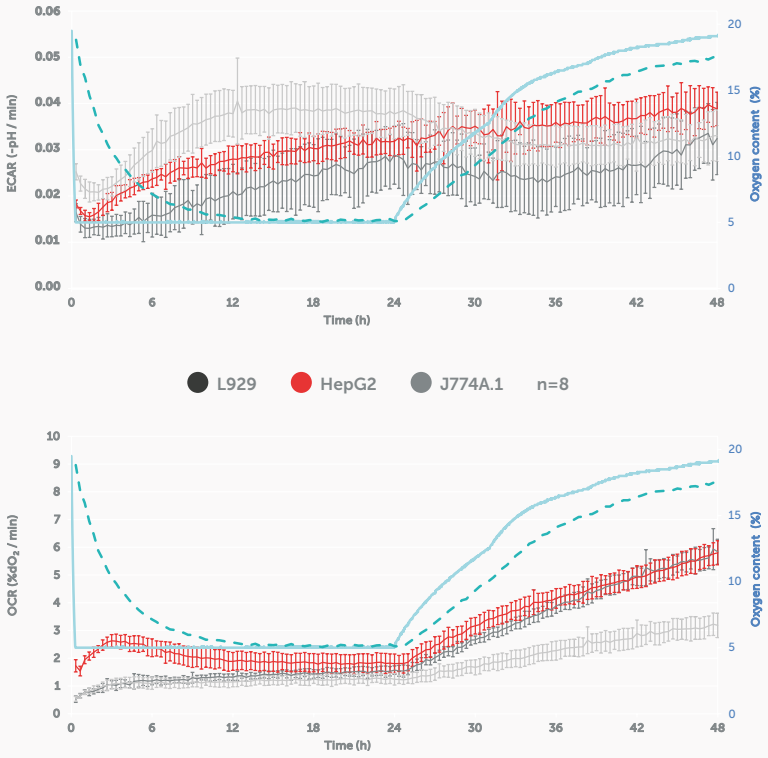
Continuous microscopic imaging by the CYRIS® analysis platform allows the detection of morphological changes in the cells, as shown in the pictures.
Prior to FCCP treatment (0h to 12h), the cell density increases steadily. With FCCP treatment (12h to 24h), the cells become flatter, intracellular precipitations appear, and the cell density remains constant. With the washout (24h to 34h), the number of intracellular precipitations slightly decreases, and the cells lose their flatness.

HYPOXIA ASSAY

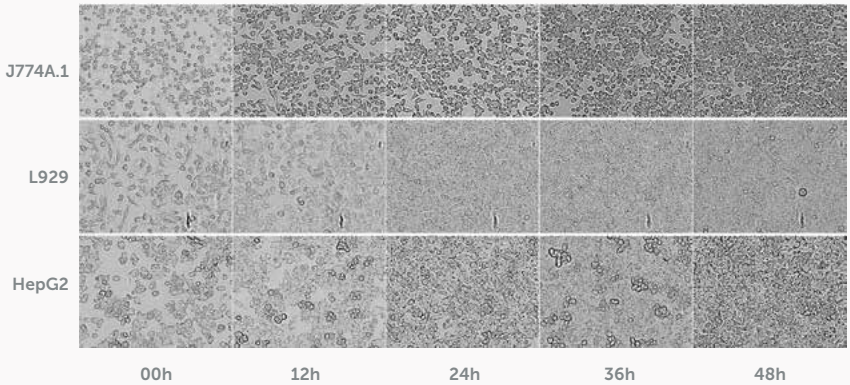
This assay shows the ability of the CYRIS® flox analysis platform to investigate cells under controlled hypoxic and re-establishing normoxic conditions.
Here we investigate three different cell lines (L929, HepG2, J774A.1) in parallel. The test settings include the monitoring of the OCR, ECAR, and microscopic images of the cells for 24h under 5% atmospheric oxygen content and for an additional 24h under an atmospheric oxygen content passively re-establishing to normoxia. During the experiment, the oxygen content in the media is calculated based on the raw data of the dissolved oxygen sensor.

The results show that on the metabolic level, all cell lines deal differently with the limited support of oxygen. The mouse macrophage cell line J774A.1, in particular, switched its metabolism to strongly increased acidification (glycolysis-driven) during hypoxia and reduced the acidification when the oxygen was not limited anymore. In contrast, the HepG2 cells showed a forced limitation of their oxygen consumption and a high amount of acidification during hypoxia, but the acidification did not decrease when the oxygen limitation was removed.

The microscopic images show a good viability and steady growth of all cell lines during the whole experiment despite the low oxygen phase in the first 24h.



OCR and ECAR of all cell lines under hypoxic and re-establishing normoxic conditions. The blue lines represent the oxygen content in the atmosphere (continuous line) and in the media (dotted line). The cell lines are reacting to the limited oxygen availability with an increased acidification, followed by an increase in oxygen consumption when the oxygen limitation is removed. However, all cell lines show a different expression of this behavior due to their own metabolic state.



PROGRESSION OF CELLULAR MORPHOLOGY

Continuous imaging allows the morphological behavior of all cell samples to be traced without gaps. All investigated cell lines show a normal morphology and a steady increase in cell density due to growth.

CYRIS as a service

LET'S DO SCIENCE

Test INCYTON® assay development services and discover if our device is suitable for your research needs.
Our cell-biology experts will design and perform your cell-based assay on our platform, and you will get significant data on cell viability and metabolic kinetics.*

Contact us at **caas@incyton.com**

REACH US

Contact us to talk about your research needs. Discuss with our scientists the option of developing a custom-tailored cell-based assay or using a ready-to-use one.

LET´S DO SCIENCE!

We define together research project aims, goals, resources and timing. Our experienced staff will perform your assay on CYRIS flox and review milestone progress with you.

RECEIVE A DETAILED REPORT

At the end of the experimentation phase, we provide you with a detailed project summary, containing protocol technical details, output data and recommendations.

DISCUSS THE RESULTS WITH US

The project outcomes will also be presented during a virtual or physical meeting. We will discuss results, identify recommendations for future research and share advice with you.

OUR OFF-THE-SHELF CELL-BASED ASSAYS AT OUR LABORATORY*

Quality results in far less time

CELL CHARACTERIZATION

- Mitochondrial stress test
- Glycolytic stress test
- FCCP-Uncoupler assay (short / long)**

TOXICOLOGICAL INVESTIGATION

- Toxicology assay (short / long)**
- Hepatotoxicity assay (short / long)**

VIRAL INVESTIGATION

- Viral infectivity test

ONCOLOGIC RESEARCH

- Migration assay
- Hypoxia assay
- Chemotoxicity assay (short / long)**

* Only available in Europe
** Optional with washout

CYRIS® FLOX

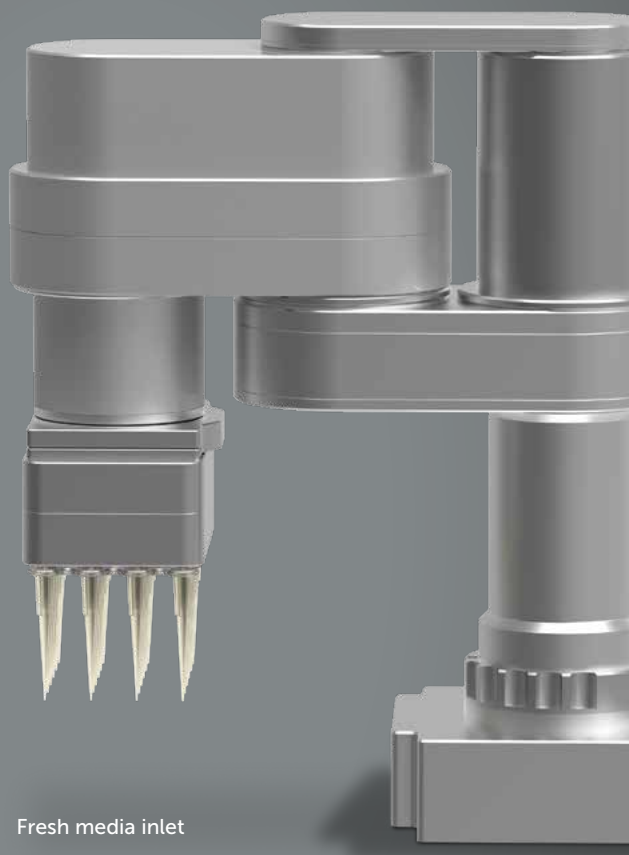
The comprehensive multisensor cell analysis platform, with label-free, high-accuracy and high-reliability monitoring.

HOW IT WORKS

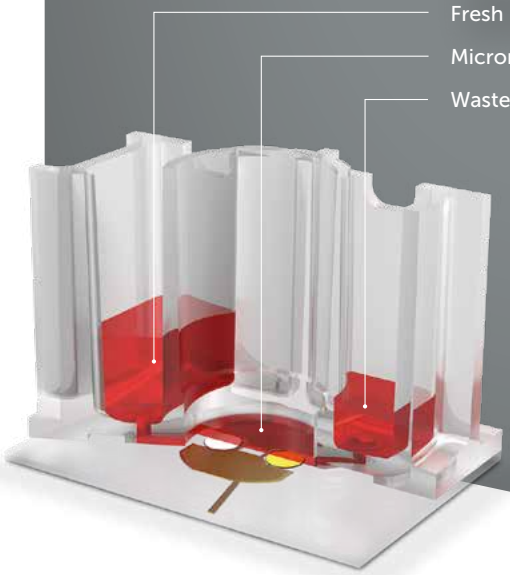
During the measurement, living adherent cells are cultivated and treated in the 24 independent test chambers of a special sensor-equipped microtiter plate (sensor plate), located in a temperature- and atmosphere- controlled climatic chamber.

A fully automated pipetting robot supplies and disposes of media and drugs in all 24 test chambers. This life support fluidic system allows the cells to be monitored and treated in the sensor plate for days up to several weeks in vital conditions and free of disturbance and contamination.

All measurements and treatment protocols in the sensor plate are individually configurable and are monitored online and in real time.

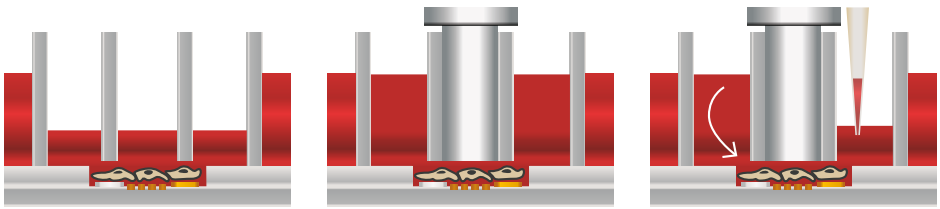


Highly compact SCARA robotic arm with 4 degrees of freedom for accurate pipetting



THE WELL

3 chamber fluidic system to ensure constant nutrition for cells, embedded with 3 sensors (pH, pO_2 and impedance) as well as a dedicated microscopy window to collect the maximum data per experiment



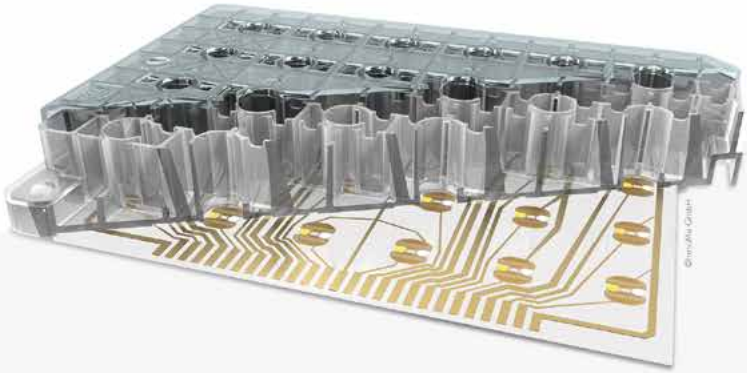
1. Cell seeding in the sensor well plate
2. Creation of microreaction chamber by special lid
3. Generation of microfluidic flow by pipetting robot

20x objective with ultra-fast and high precision focusing

CONSUMABLES

We can provide you with three different types of well sensor plates

	Fully equipped - FE | sensor plate 24	**Metabolic - ME | sensor plate 24**	**Imaging - IM | sensor plate 24**
Dedicated microscopy window	○	○	○
Highest data output	+++	++	+
Optochemical sensors for pO2 and pH measurement	○	○	
Gold IDES Structure on glass surface for cellular impedance analysis	○		



TECHNICAL DETAILS

General technical data		
Dimensions (W/H/D)	mm	730 x 1200 x 650
Weight	kg	230
Electrical supply	VAC / Hz	230 ± 10 / 50-60

Environmental conditions for storage and operation		
Temperature range	°C	5 - 40
Relative humidity, maximum	% rH	80

Incubator		
Setting temperature range	°C	+18 to +50
Setting temperature step	°C	0.1
Setting relative humidity range	% rH	30 - 90
Setting O2 range	%	1 - 21

Robot		
Pipetting volume	µl	5 - 200
Amount of channels / tips	-	24

Microscope imaging system		
Objective magnification	x	20
Z – drive type	-	VoiceCoil
Focus range / resolution	µm	6800 / 0.002
Camera resolution	MPx	12.3
Pixel size	µm	3.45

Cellular impedance		
Throughput (wells per second)	1 / s	14
Measuring frequency	kHz	10
Measuring range impedance Z	Ω	10 - 5000
Measuring range resistance R	Ω	10 - 5000
Measuring range capacity C	nF	0.3 - 3000
Measuring error	%	< 2

pH and pO_2 single channel measuring device		
	pO_2	pH
Measuring range	0 - 50 %	6.0 - 8.5 pH
Resolution	± 0.4 % pO_2	± 0.05 pH
Signal drift	0.2 % / week	0.1 pH / week
Accurateness	± 1.0 % at 20.9 % pO2	± 0.2 % at pH7

CYRIS® ACE - Software Specifications	
Applications	Create & analyse assay result files
Operating Systems	Windows 10 and above

HOW TO GET A CYRIS® FLOX IN YOUR LAB?

If we have piqued your interest, here are some ways you can have a CYRIS device in your lab

- Financing through our partner:
  Offers you full cost control and manageable terms through variable down payment and final installment options.
- Monthly rental:
  You can customize your CYRIS® rental needs in 3 month increments and pay a monthly fee for the duration of the service.
- Pay per use:
  If you want to use a CYRIS® for a short and limited time, you can have a device billed on a per experiment basis, in which the costs for consumables are already included.

QUESTIONS?
Just ask us about the perfect way for you.

SCIENTIFIC COOPERATION

If you have a research idea or drug discovery project where CYRIS® could help you.
Let us know – we are always looking for scientific cooperation.

CONTACT

CYRIS® flox – Metabolism and morphology at a glance

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