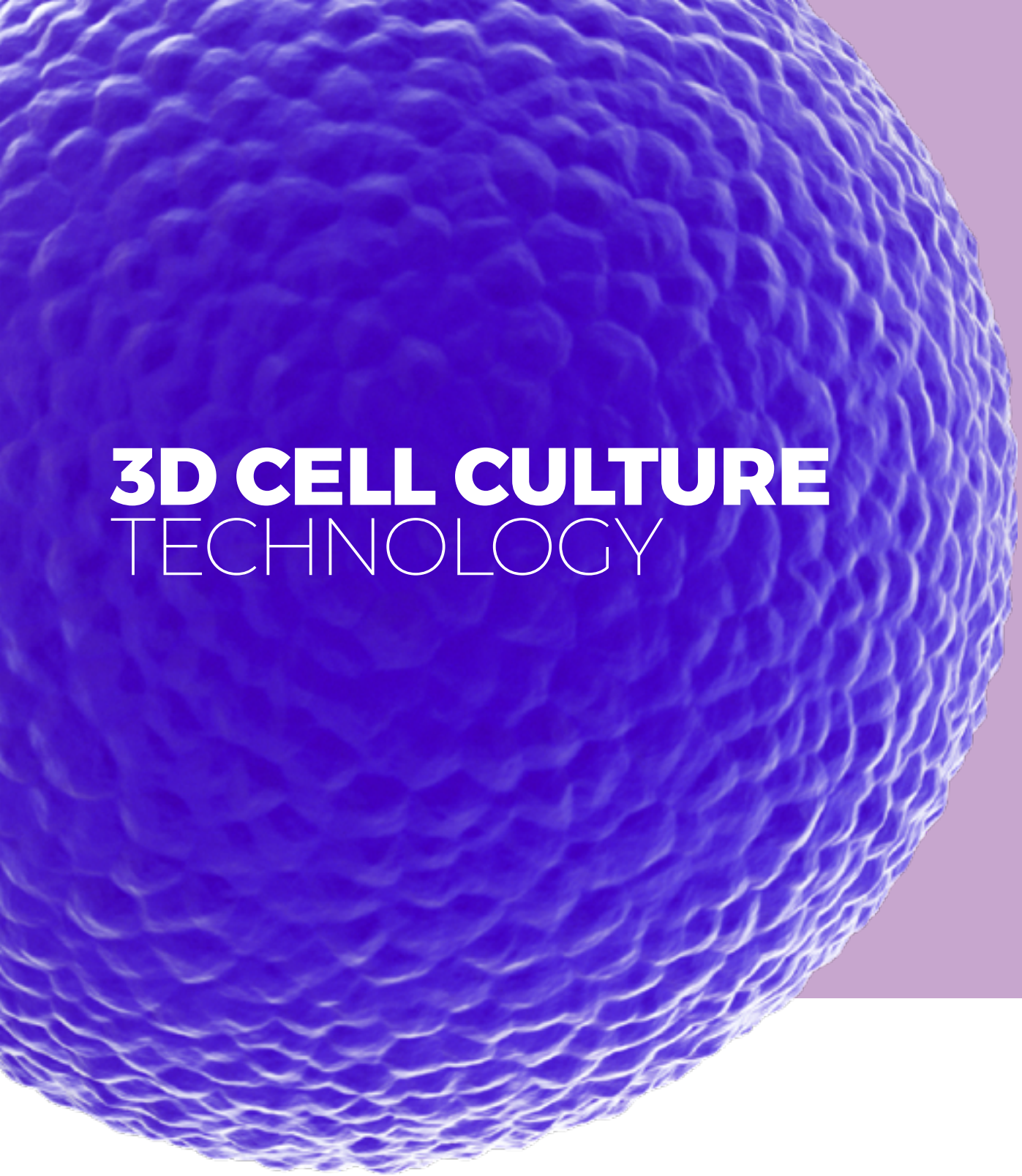


3D CELL CULTURE TECHNOLOGY

www.celvivo.com

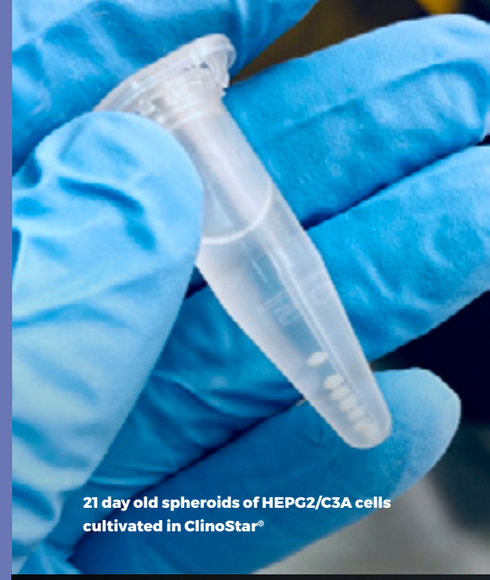
CELVIVO
STRESS-FREE 3D™

UNIMED 騰達行 0800-211-819

ClinoStar® is a cutting-edge system that provides scientists with superior growth conditions, to implement spheroid and organoid culture in their labs.

3D CELL CULTURE
INCREASE THE PERFORMANCE OF YOUR CELL LINE

The ClinoStar® was developed because we needed cell cultures with in vivo functionality for our research. The system is easy to operate and create abundant and reproducible 3D spheroids and organoids



21 day old spheroids of HEPG2/C3A cells cultivated in ClinoStar®

ClinoStar® creates an environment that promotes longevity and creates uniform cell constructs

The ClinoStar® system is advanced bioreactor platform that creates an environment, which promotes growth, maintenance and functionality of large 3D tissue mimetic structures, including spheroids, organoids and other cell aggregates.

It provides conditions which allow cells to develop functionality that closely mimic conditions in the intact organism.

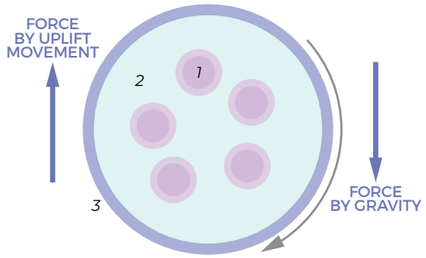
CELVIVO
STRESS-FREE 3D™

CLINOSTAT FOR CELL CULTURE

The system employs a clinostat principle (a rotating bioreactor) to keep the cells in suspension by counterbalancing the gravitational forces.

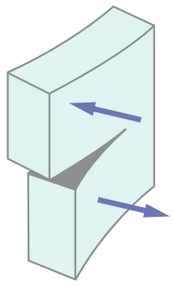
This approach has the advantage that the spheroids and organoids formed, are exposed to very low shear forces and have good gas and nutrient exchange. Low shear forces, combined with active diffusion, are key parameters for cells to develop into functional 3D constructs.

CLINOSTAT PRINCIPLE



1) cells; 2) culture media; 3) culture vessel

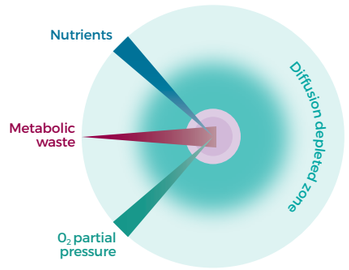
LOW SHEAR FORCES



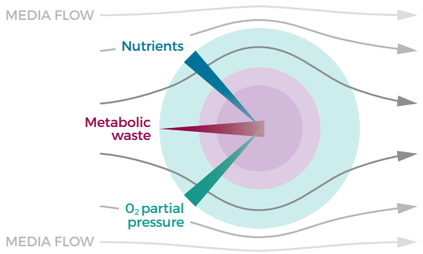
The smooth rotation (2.5-30 rpm) created by the clinostat matches the gravitational force, ensuring that spheroids or organoids are kept in a ‘stationary orbit’. In this stress-free environment, cells self-organize and maintain or re-establish their architecture and functionality so that they resemble their parental tissue.

ACTIVE DIFFUSION

Passive diffusion
175 µM radius spheroid



Active diffusion
450 µM radius spheroid



Inside ClinoReactor the spheroids or organoids will experience continued media flow, abolishing the diffusion depleted zone observed with static cultures. This active diffusion across the cell conglomerates, allows for better nutrient and gas exchange, essentially allowing the spheroids and organoids to develop to the necessary size and to mimic native cytoarchitecture and display physiological attributes of the native tissue

FEATURED FRIENDS

Our featured friends are a group of experienced scientists that have used the ClinoStar® in their own research.
See what they think or read their publications.

”We are adapting all of our projects using spheroids. They model more accurately every aspect of cell physiology compared to canonical flat cultures, and this has allowed us to eliminate part of animal testing from our workflow. We are really impressed by how simple it is to maintain and prevent contaminations of these spheroids for both experienced and inexperienced users.”

Simone Sidoli - Assistant Professor
Department of Biochemistry at the
Albert Einstein College of Medicine

To find out more about Simone
and his research, scan the QR Code

”Spheroids created with CelVivo system have become an indispensable tool in my research combining proteomics and lipidomic to understand the role of protein oxidation and redox imbalance in cell physiology induced by drugs and xenobiotics.”

Adelina Rogowska-Wrzesinska - Associate Professor
Department of Biochemistry and Molecular Biology,
University of Southern Denmark

To find out more about Adelina
and her research, scan the QR Code

”Using clinostat-based 3D cultures enables much longer treatment and experimental windows, in an in vitro format, while obtaining data with physiological relevance. Unlike with animal models, multiple daily samplings from the same bioreactor is possible, with no ethical implications. I believe this is an ideal approach.”

Chrisna Grouws - Associate Professor
Pharmaceutical and Biomedical Pharmaceutics,
North-West University

To find out more about Chrisna
and her research, scan the QR Code

CLINOSTAR® FEATURES & BENEFITS

The ClinoStar® is a advanced CO_2 incubator with six independent motors (clinostats), which each can hold a bioreactor (ClinoReactor). The system is operated using a tablet* with pre-installed software that permits control of the temperature and CO_2 level of each ClinoStar® independently. Six camaras located opposite to the motors enable video surveillance of the cultures without disturbing the environment.

Push to open
For convenient hands-free opening and reduced contamination risk.

Adjustable light
Front and backlight can be adjusted to obtain crystal clear images.

Small footprint
Fits anywhere, even in your laf-bench.

Uniform environment
The large heating element and fan ensure an equal distribution of heat and CO2 across the chamber.

Connectivity options
An ethernet connection allows direct internet access, for example to receive software updates via the control unit.

Decontamination
Automatic UVC-decontamination cycles.

**Supplied with the starter pack.*

CELVIVO STRESS-FREE 3D™

DIMENSIONS
SEE PAGE 15

Remote Control
Control, adjust and monitor your cultures. One Tablet can control up to 50 units.

Software over the air
New features and tools are implemented by software updates.

Live Camera Feed
No need to open to the door. Track your culture through the 5MP camera.

x6

Enable 6 individual cultures
The six motors can be individually adjusted.

CLINOREACTOR FEATURES & BENEFITS

The ClinoReactor is a fixed 10 mL culture chamber, supplied sterile in a sealed package. The blue humidification beads provide a humid atmosphere. This eliminates the need for additional water in the incubator and this significantly reduces the risk of unwanted infections.
To prevent cell, protein and drug absorption, the bioreactor is made from low binding polypropylene and polystyrene. The optically clear polystyrene end wall enables direct microscopy of the spheroids or organoids without needing to open the ClinoReactor.

Fixed 10 mL cell culture chamber
Can contain and maintain over 350 mature cell constructs with over 80000 cells in each.

Low bind surface
Polypropylene and polystyrene hydrophobic surfaces surfaces ensure a low adhesion and adsorption of molecules.

Click-on
Simple click-on system for easy placement and removal of ClinoReactors in ClinoStar®.

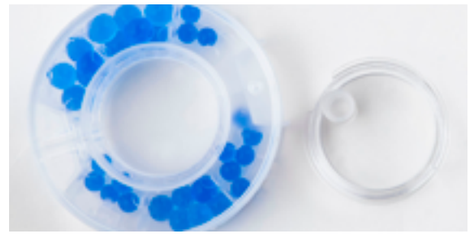
Unique airflow and filter system
The 0.2 µm filter protects the cultures from infection while still allowing CO_2 and O_2 exchange.

Free standing
Integrated feet at the base of the ClinoReactor allow it to stand vertically for easy media exchange.

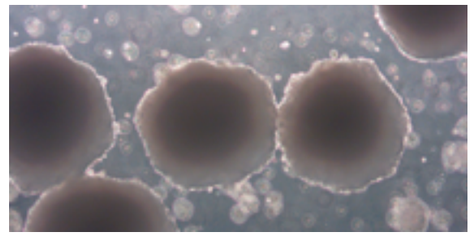
CELVIVO STRESS-FREE 3D™

SEALED, WRAPPED AND READY TO USE!

PRODUCT DATA SEE PAGE 15



Petri dish accessibility
Scan the QR Code to see the functionality of the petri dish opening.



Optically clear lid
ClinoReactor can be placed directly under the microscope.

Closed environment
Contained humidification system maintain a constant volume in the chamber, and limit the risk of infections spreading.

Easy media exchange through the top port
The media can be exchanged for all cell constructs within a minute.

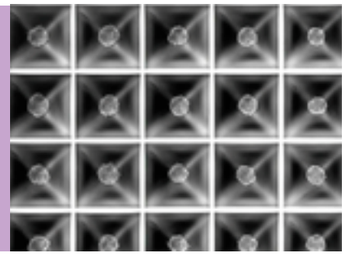
HOW TO WORK WITH CLINOSTAR®

Culturing spheroids and organoids is dependent on many factors. With the ClinoStar® system, we have created a flexible system to generate spheroids and organoids in a reproducible fashion. The initial cell aggregation can be performed in several ways depending on cell and sample types. Downstream processes are easy to control, the infection risk is minimised so that cultures can be kept for years in the ClinoStar® with a minimum of effort.

Spheroid and organoid development

1. Select aggregation method

Following 2D cell expansion or tissue disaggregation, the cells can be seeded in the ClinoReactor with one of three approaches: as a single cell suspension, or following initial aggregation preformed using a hydrogel or in micropattern plates.

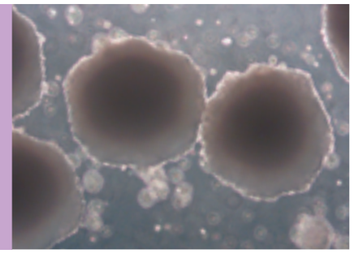


2. Transfer to ClinoReactor

When starting with the ClinoStar® system, you can use the cell culture media and supplements that you are used to. Specialised media, scaffolds, ECM substitutes or growth factors are not needed.

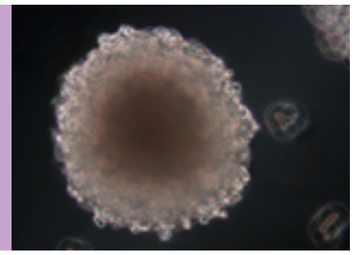
3. Cultivate in ClinoStar®

In the cultivation phase, cell culture media is routinely renewed, and irregular constructs are removed from the ClinoReactor to ensure uniform growth. Check that your constructs are functional against an in vivo benchmark that can be assayed in vitro.



4. Mature the constructs

When the cell lines have recovered their functionality, they are considered mature and ready for experimentation. This occurs after 18 days for HEPG2/C3A cells. At that time, each ClinoReactor will hold around 350 spheroids, each containing about 82,000 cells.



BUILT TO AVOID CONTAMINATION

Infections are one of the main challenges in cell culture and it can cause significant delays.

A series of features in the ClinoStar® system have limited the cell cultures contact with their surroundings, removed unnecessary contamination points and made it easy to clean.

EASY CLEANING

Corner-less design
Easy cleaning throughout the experiment

UV decontamination
Run regular sterilisation cycles

Glass and smooth surfaces
Disinfection and cleaning with 70 % ethanol or equivalent

REDUCED EXPOSURE

Push to open
Use your elbow to keep your hands clean

Camera monitoring
Clear view without opening

Solidified humidification
Separate humidification system in each ClinoReactor

PROTECT AND CONTAIN

Double wrapped
Open directly into the sterile workspace

Easy clean ClinoReactor
The collar is easily disinfected with ethanol

Infections are kept out.
The closed design ensures infections are not spreading

CLINOSTAR® PROMOTES CELL FUNCTIONALITY

For a model to have the highest value, it must provide an accurate representation of human physiology *in vivo*.

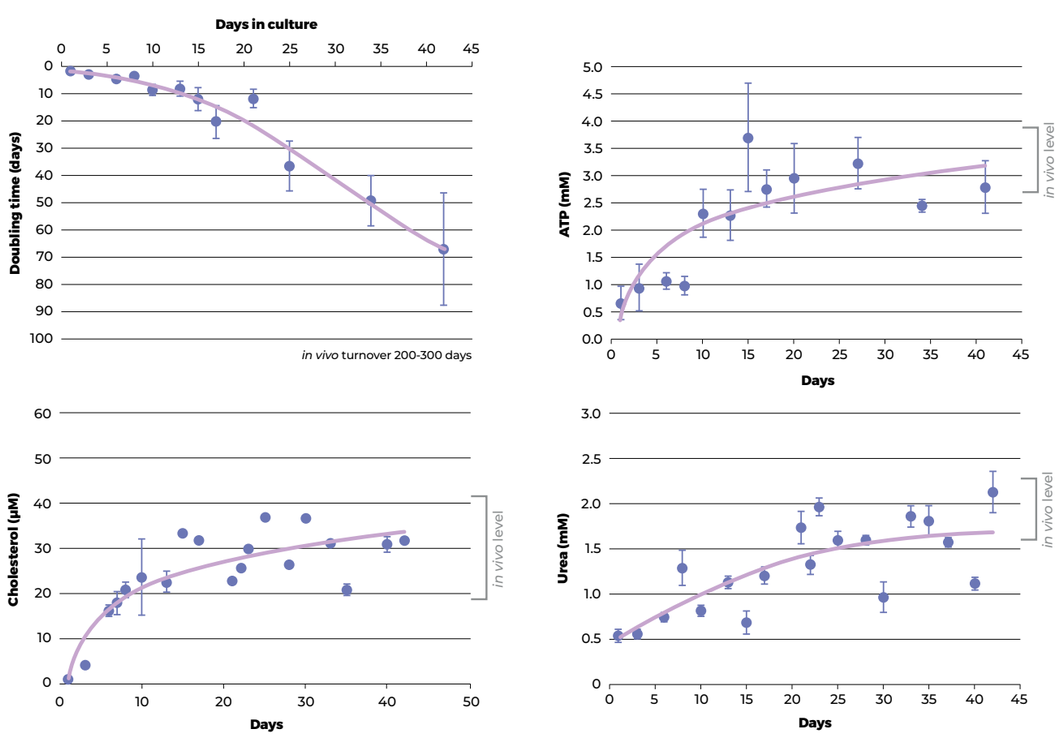
Many cells, when grown in 2D cultures have doubling times of 1-2 days. In contrast, cells in tissues for example the liver, have a doubling time of 200 – 300 days (the rate needed to maintain the tissue). In 2D cultures, HepG2/C3A cells – a cell line derived from a hepatocellular carcinoma has a doubling time of about 1 day (top left figure). If those cells are grown as spheroids in a ClinoReactor, their rate of proliferation slows dramatically so that after 42 days in culture , their doubling time is about 70 days (much closer to the situation *in vivo*).

We investigated three physiological functions seen in the liver and compared the performance of spheroids of different ages with that of the same number of cells in an adult human, Figure 1.

Both cholesterol and urea* are primarily synthesised in the liver, while ATP is synthesised in every living cell. In all three situations, we found that it took about 18 days for the rate of synthesis to increase to levels seen *in vivo*. After this time, the rates stabilised – at least for the next 24 days (and probably much longer). This provides a large window for experimentation where spheroids are at a metabolic equilibrium, Figure 1.

For this reason – and for the sake of convenience, we therefore recommend that – for the C3A cell line – spheroids are cultivated for 21 days if one wishes to mimic *in vivo* physiology. Other cells (whether primary or pluripotent cells or cell lines) may reach this equilibrium at different times and so it is important to benchmark against the *in vivo* performance (Wrzesinski et al. 2013).

Figure 1. *Charataristics of a HepG2/C3A cells cultivated in ClinoStar®. Doubling time, ATP content, cholesterol and urea production have been evaluated at multiple timepoints up to the endpoint at 42 days (Wrzesinski et al. 2013). concentration units are denoted: [conc]/day/g of soluble protein.*



*Interestingly, urea production occurs via the alternate pathway because two genes (ornithine transcarbamylase and arginase I) of the urea cycle have been lost from C3A cells.

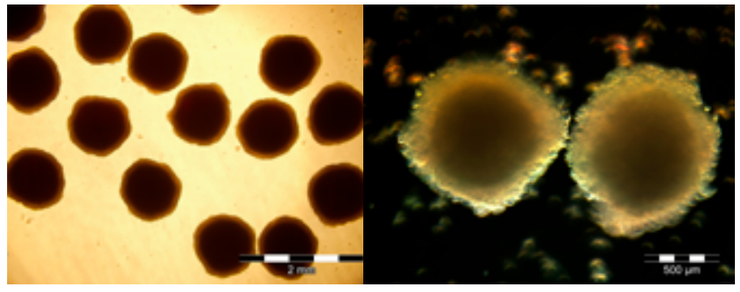
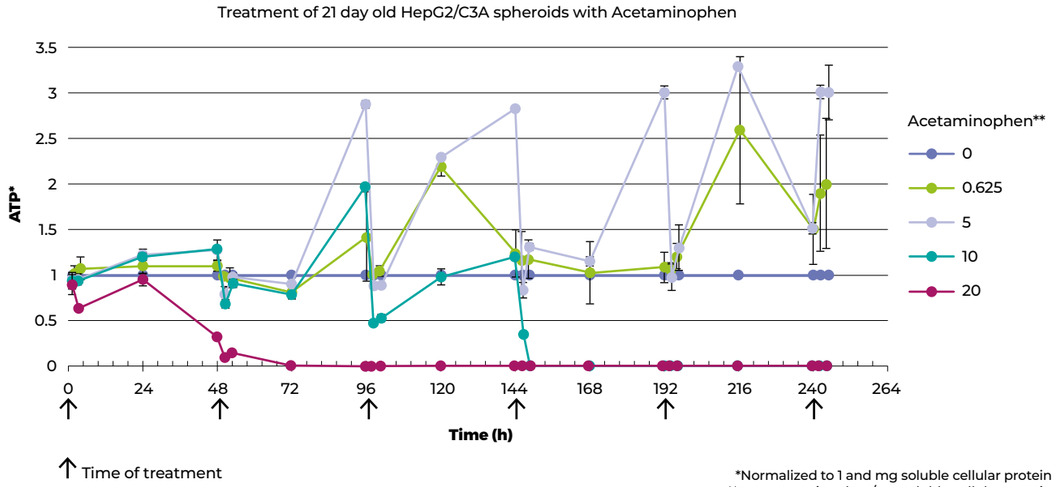


Figure 2. *HEPG2/C3A spheroids seen in phase contrast and dark field microscopy. Note the low variability between the spheroids. Note also, that light cannot penetrate the spheroids, so it is necessary to choose assays that are relevant for tissue biopsies and not monolayer of cells.*

Figure 3. *Repeated APAP (Paracetamol) treatment of 21 days old HepG2/C3A spheroids with subsequent evaluation of ATP content at multiple concentrations. Black arrow notes treatment point. (Fey, Korzeniowska and Wrzesinski, 2020).*



*Normalized to 1 and mg soluble cellular protein
**mg acetaminophen/mg soluble cellular protein

One of the roles of the liver is in the detoxification of compounds. To investigate whether this can be modelled in vitro, 21 day old HEPG2/C3A spheroids were treated with various concentrations of acetaminophen (APAP; paracetamol) at 48 hours intervals for 10 days (black arrows). The effect on the cells was evaluate via ATP content (CellTiter-Glo assay, Promega). The spheroids treated with highest dose (20mg APAP / mg soluble cellular protein) lost their viability already after first treatment (red line –acute toxicity).

Multiple treatment of spheroids with 10mg APAP / mg soluble cellular protein (half of the acute toxicity dose) lids to graduate lost of cellular viability (blue line – chronic toxicity). Halving the dose again illustrates that the HEPG2/C3A spheroids can respond to and recover from the treatment. Even at physiological doses (for example for treating a headache), the HEPG2/C3A spheroids still show a response and recover behavioral (0.625 mg/mg, green line). Similar response and recovery patterns have been seen for six drugs tested acetaminophen, amiodarone, diclofenac, metformin, phenformin and valproic acid) and illustrate that HEPG2/C3A spheroids can be used for the determination of repeated-dose drug toxicity, eliminating the need for using animals for this purpose (Fey et. al., 2020)(Wojdyla et al. 2016).

Resources:

Wrzesinski et al. 2013
Scan to access the article

Fey et al. 2020
Scan to access the article

Wojdyla et al. 2016
Scan to access the article

List of publications
Scan to access the complete publication list

BOOK YOUR CLINOSTAR® DEMO

Are you ready to make the transition to functional 3D cell culture?

Book a ClinoStar® demonstration now and try cultivating spheroids or organoids in your own lab with ClinoStar®.

Scan the QR code to access our contact page.

CONTACT US

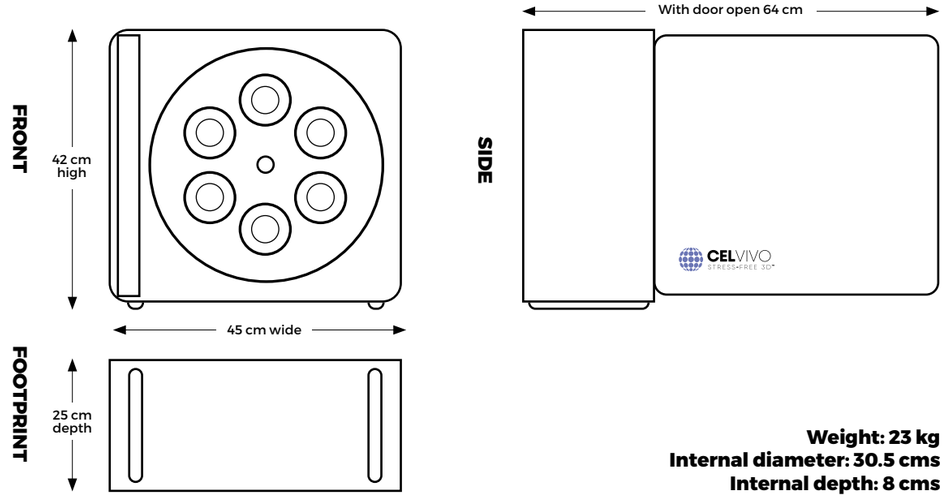
If you have any questions about our products, please ring our customer helpline

Call (+45) 70 228 228

PRODUCT DATA

CELVIVO STRESS-FREE 3D™

ClinoStar® measurements



Weight: 23 kg
Internal diameter: 30.5 cms
Internal depth: 8 cms

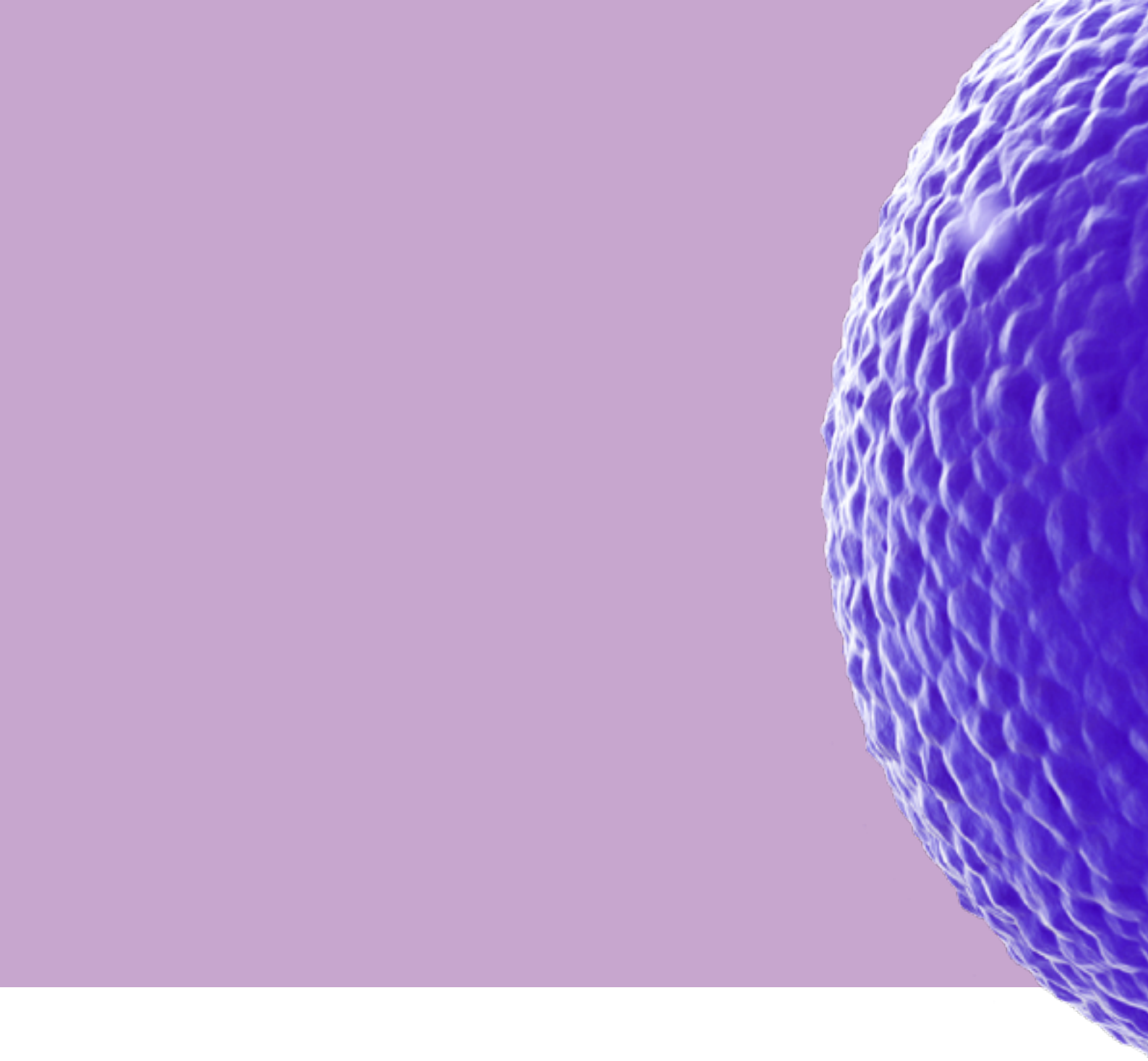
ClinoStar® specifications

Door	
Open mechanism	Push – click – swing open
Close mechanism	Push to close - click
Axles	
Capacity	6 axles
Speed range (rpm)	0 - 100
Speed Accuracy	±1 %
Direction	Clockwise or anti clockwise
Control	Independent
Temperature data	
Temperature range	From 6 to 20 °C above ambient
Temperature accuracy	± 0.25 °C
CO_2-data	
CO2 range [Vol.-% CO_2]	0 - 10 %
CO2 measurement	IR
CO2 calibration	Factory calibrated for 10 years
Monitoring	
Cameras	6 (placed opposite to each axle)
Camera resolution	5 Megapixel
Lighting	Front and back LEDs for each axle
Decontamination	
Incorporated method	UV-C LED 300 mA
Time	User activated (2 hours runtime)
Controller	
Device	Tablet
Communication method	Wi-Fi, Ethernet
Screen size	10,1"
Screen resolution	1920 x 1200
Units to control	50

Safety	
Paused while door is open	UV-C emitting LED, fan and CO_2
Connectors	
USB	Back and front (in door)
Network	Ethernet (RJ-45) Ethernet port provide 1500V insulation
CO_2	Ø8mm with last-resort filter
Footprint	
Space saving configuration	Up to 3 units can be stacked on top of each other (the stacking bar must be used to increase their stability)
Electrical data	
Rated Voltage [V]	100 - 240 V
Power frequency [Hz]	50 - 60 Hz
Nominal power [A]	1.8 A
Power cord length	2 metres
Appliance Class	Class I equipment
Pollution Degree	2
Overvoltage category	II

ClinoReactor specifications

Catalogue #
10004-12
Size
12 x ClinoReactor with 10 mL culture chamber
Description
ClinoReactor is made from polypropolene and polystyrene. Each Supplied with sterile 25 mL water for rehydration of water beads. 10 days use.



CelVivo ApS
Denmark
Telephone: (+45) 70 228 228
info@celvivo.com

CELVIVO
STRESS-FREE 3D™