3D CELL CULTURE TECHNOLOGY

www.celvivo.com

CELVIVO
STRESS-FREE 3D™

PhileKorea
Specialized in Medical & Bio Science since 2000

With ClinoStar®, we built 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

ClinoStar® was built because we needed a better model for our research. The success criteria was a system which was easy to operate and could create abundant and reproducible models with *in vivo* functionality.

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 a 3D 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 enables conditions, which allow cells to develop functionality that closely mimics conditions in the intact organism.

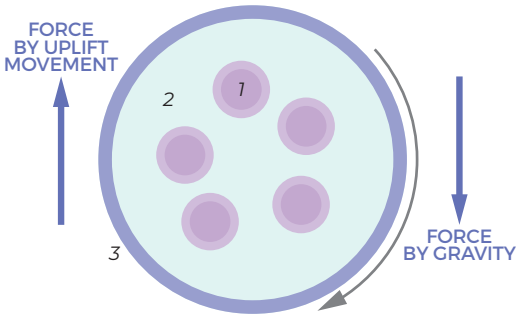
CELVIVO
STRESS-FREE 3D™

CLINOSTAT FOR CELL CULTURE

The system employs a clinostat and a rotating bioreactor to keep the cells suspended by counterbalancing the gravitational forces.

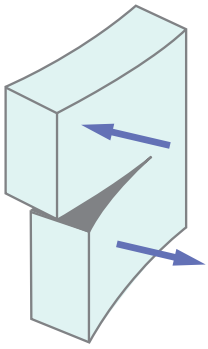
This approach has the advantage that the spheroids and organoids formed, are exposed to very low shear forces and have good gas and nutrient exchange. Low shear forces, combined with active diffusion, are key parameters for cells to develop into functional 3D constructs.

CLINOSTAT PRINCIPLE



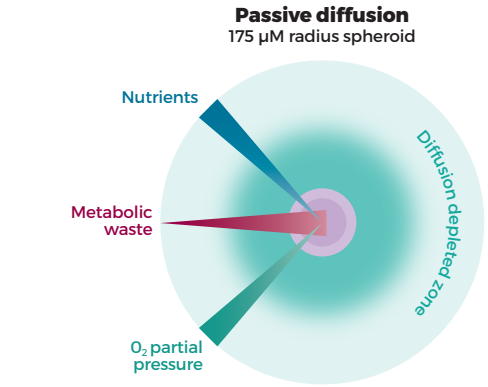
1) cells; 2) culture media; 3) culture vessel

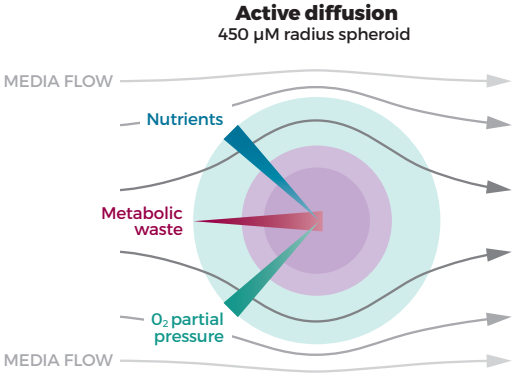
LOW SHEAR FORCES



The gentle rotation (2.5-30 rpm) created by the clinostat, ensures the spheroids or organoids are kept in statical orbit with low shear stress introduced. In this stress-free environment, the cells have time to self-organize and maintain or re-establish their architecture and functionality to resemble their parental tissue.

ACTIVE DIFFUSION





With the clinostat principle, 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® prototype 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 premium class 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 and low variability of heat and CO_2 across the chamber.

Connectivity options
An ethernet connection allows for direct internet access or 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.

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

CLINOREACTOR FEATURES & BENEFITS

ClinoReactor is a bioreactor with a fixed 10 mL culture chamber, that is supplied sterile in a sealed and wrapped package for convenient use and to limit the infection risk. The humidification beads supply water for the culture chamber, which eliminate the need for water in the incubator. To prevent cell, protein and drug absorption, the bioreactor is made from low bind polypropylene and polystyrene. The optically clear properties of the polystyrene enables microscopy of the spheroids or organoids in a closed environment.

Fixed 10 mL cell culture chamber
Can contain and maintain over 350 mature cell constructs.

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

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

Unique airflow and filter system
0.2 µm filter protect the cultures from contaminants while gas exchange is maintained, enabling use of bicarbonate buffered media.

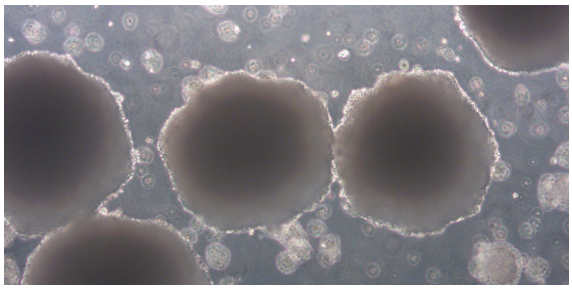
Free standing
Integrated feet at the base of the ClinoReactor provides a stable foundation when exchanging media.

SEALED, WRAPPED AND READY TO USE!

CELVIVO
STRESS-FREE 3D™

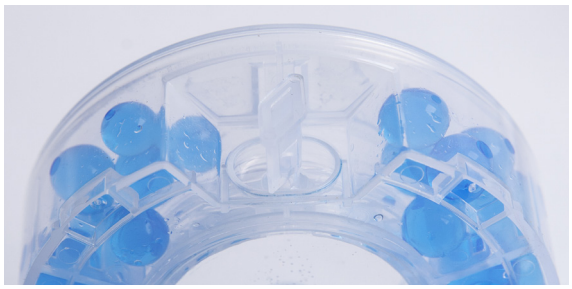
PRODUCT DATA
SEE PAGE 15

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



Optically clear lid
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
Simultaneous media exchange for all cell constructs, performed within minutes.

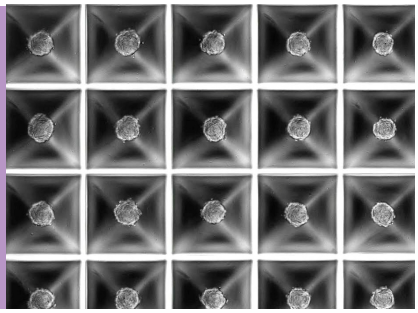
HOW TO WORK WITH CLINOSTAR®

Culturing spheroids and organoids is dependent on the cell model and sample being cultured. With the ClinoStar® system, we have created a generic system to generate spheroids and organoids in a reproducible fashion. The initial cell aggregation, can be performed in several ways to accommodate various cell and sample types, but the downstream process remains. Each step, breaks down the process of generating reproducible spheroids and organoids with ClinoStar®.

Spheroid and organoid development

1. Select aggregation method

Following 2D cell expansion, the cells can be seeded in the ClinoReactor with one of three approaches: single cell suspension or initial aggregates preformed in hydrogel or micropattern plates.

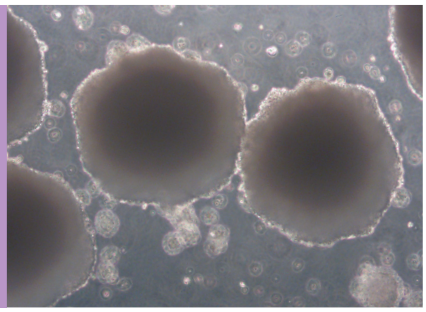


2. Transfer to ClinoReactor

In the ClinoStar® system, you can use your regular cell culture media and supplements, obviate the need for specialised media, scaffolds, ECM substitutes or growth factors.

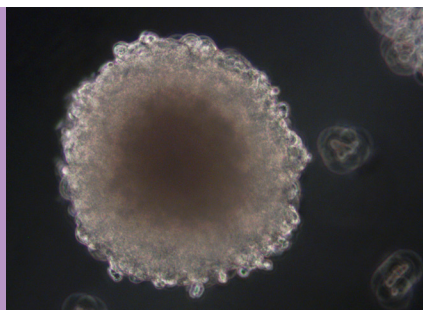
3. Cultivate in ClinoStar®

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 a parameter that can be assayed *in vivo*.



4. Mature the constructs

When the cell lines have recovered their functionality, they are considered mature and ready for experiments. For HEPG2/C3A cells each ClinoReactor will hold around 350 spheroids after 18 days of maturation.



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 though out the experiment

UV decontamination
Run regular sterilisation cycles

Glass and smooth surfaces
Disinfection and cleaning with ethanol

REDUCED EXPOSURE

Push to open
No contact with hands

Camera monitoring
Clear view without opening

Solidified humidification
Seperate 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

Infection is contained
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*.

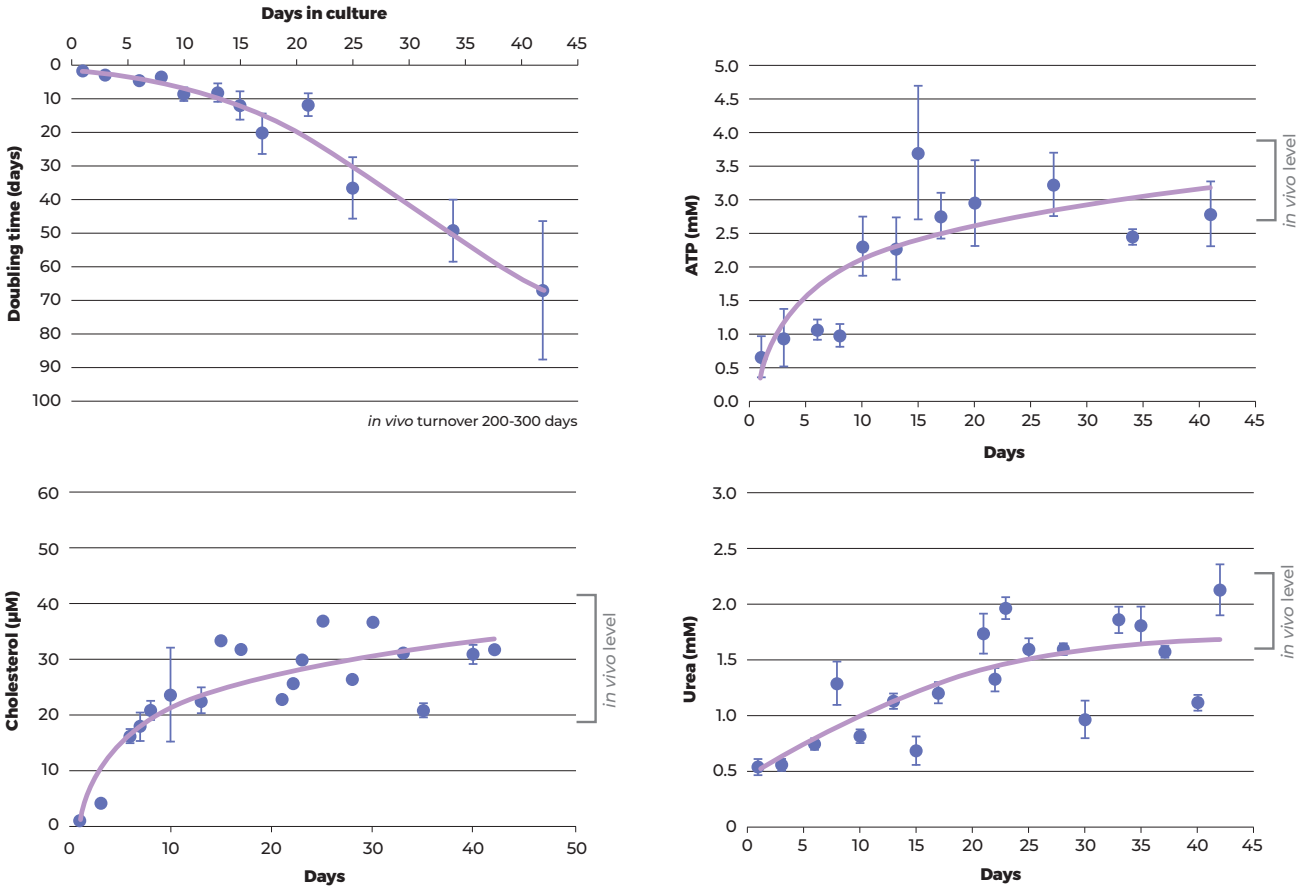
Many cells, when grown in 2D cultures have doubling times of 1-2 days. In contrast, cells in tissues for example the liver, have a doubling time of 200 – 300 days (the rate needed to maintain the tissue). In 2D cultures, HepG2/C3A cells – a cell line derived from a hepatocellular carcinoma has a doubling 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).*



*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.

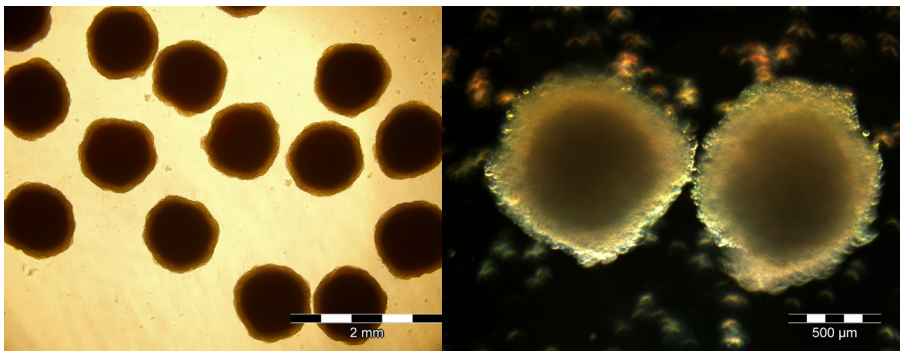
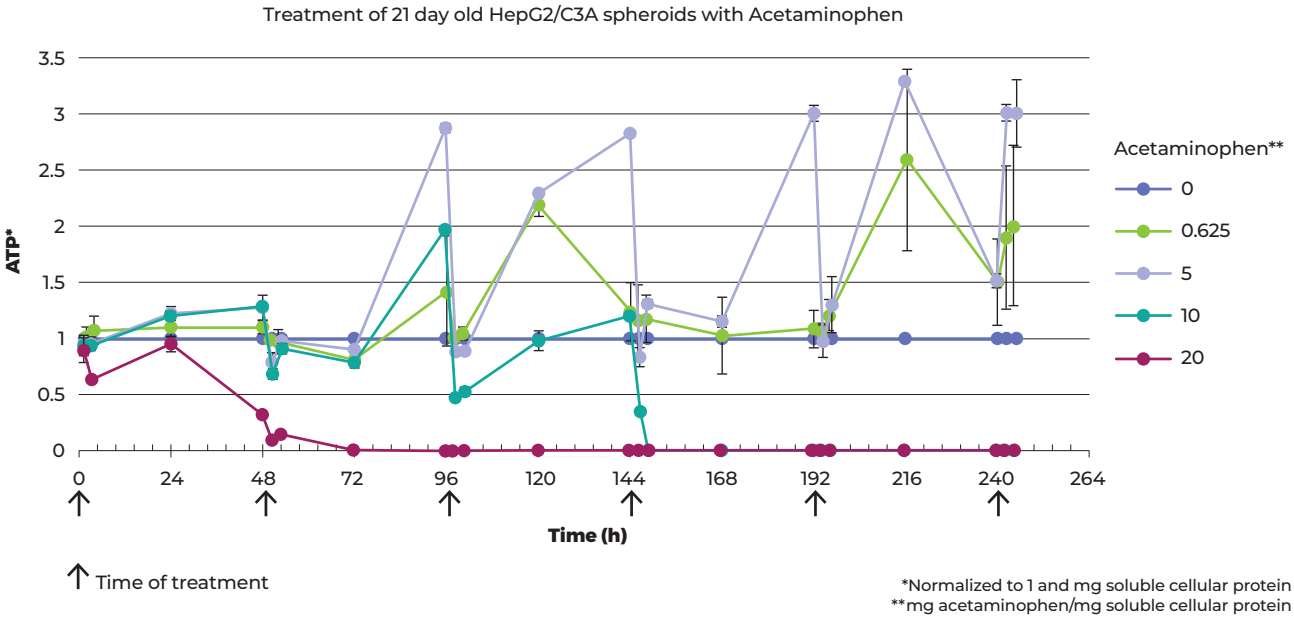


Figure 2. *HEPG2/C3A spheroids seen in phase contrast and dark field microscopy. Not the low variability between the spheroids. Note also, that light cannot penetrate the spheroids, so it is necessary to choose assays that are relevant for tissue biopsies and not monolayer of cells.*

Figure 3. *Repeated APAP 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).*



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 does of acetaminophen (APAP, also known as paracetamol) at two day intervals for 10 days (black arrows). The effect on the cells was evaluate via ATP content by the CellTiter-Glo assay from Promega. The highest dose (20mg APAP / mg soluble cellular protein) killed the cells after one dose (red line – acute toxicity).

Halving this dose illustrated that multiple doses were needed to kill the cells (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 behaviour (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, Korzeniowska and Wrzesinski, 2020).

Resources:

Wrzesinski et al. 2013
Scan the QR Code
to see the article.

Fey, Korzeniowska and Wrzesinski, 2020
Scan the QR Code
to see the article.

List of publications
Scan the QR-code to access
a 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, fill out your details and we will contact you to arrange a demonstration.

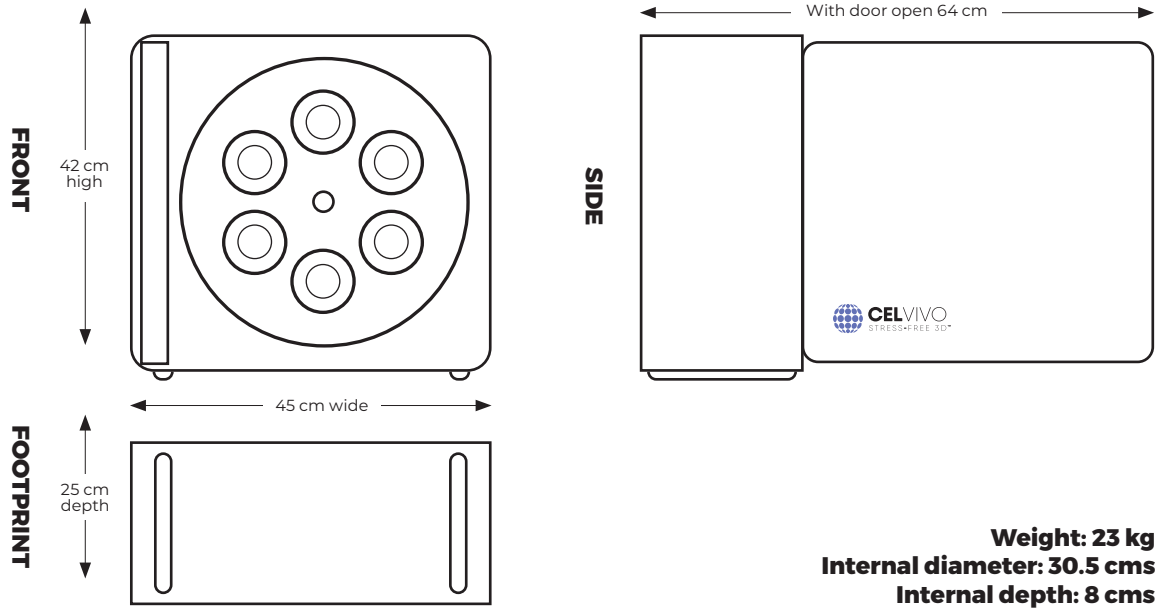
CONTACT US

If you have any questions about our products, please contact our local distributor

Telephone: (+45) 70 228 228

PRODUCT DATA

ClinoStar® measurements



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

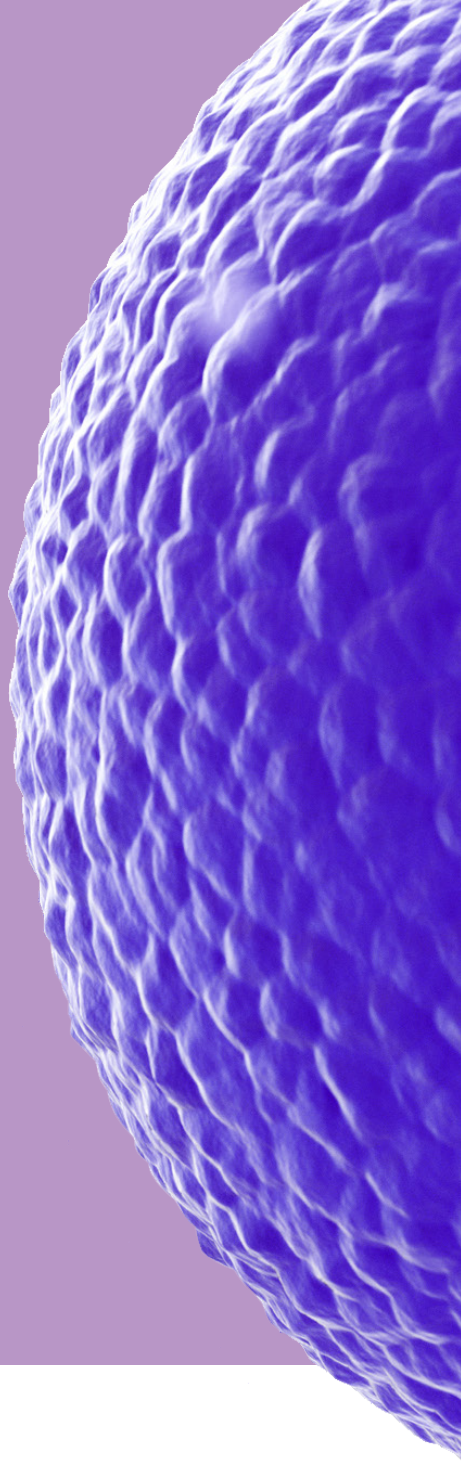
ClinoStar® specifications

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	
CO_2 range [Vol.-% CO_2]	0 - 10 %
CO_2 measurement	IR
CO_2 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)
CO_2	Ethernet port provide 1500V insulation
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. Supplied with 25 mL water for rehydration of water beads. 2 week use.



PhileKorea

1st PhileKorea, Inc. ㈜필코리아테크놀로지

(주)필코리아테크놀로지
Tel: (02)2105-7020
http: www.philekorea.co.kr
E-mail: info@philekorea.co.kr

CELVIVO
STRESS-FREE 3D™

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