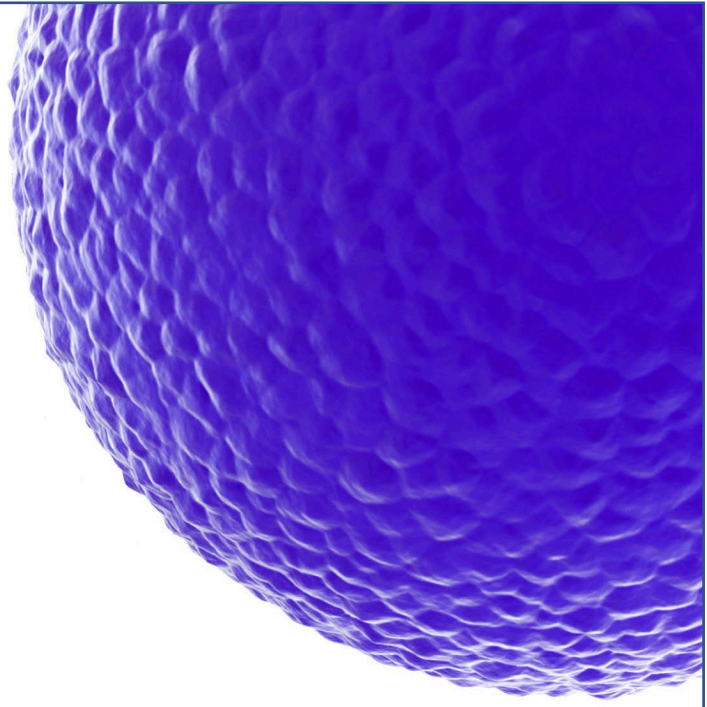


CELVIVO

STRESS-FREE 3D™

CLINOSTAR
USER & SERVICE MANUAL

IMPORTANT

READ THIS INSTRUCTION MANUAL

Failure to read, understand and follow the instructions in this manual may result in injury to operating personnel, damage to the unit and/or poor equipment performance.

Disclaimer: This equipment is for research use only.
Materials produced by the use of this equipment must not be used for diagnosis or treatment in any type or form.

All internal adjustments and maintenance must be performed by qualified service personnel.

Material in this manual is for information purposes only. The contents, and the product it describes, are subject to change without notice. CelVivo makes no representations or warranties with respect to this manual. In no event shall CelVivo be held liable for any damage, direct or incidental, arising out of, or related to, the use of this manual.

TABLE OF CONTENTS

PREFACE

We have prepared the ClinoStar to be as intuitive as possible and these instructions to help you get the most out of it, easily and safely. In this way, you can concentrate on your experiments and not have to concentrate on how to use this instrument. The ClinoStar has been manufactured to keep up with the latest technological developments and is operationally safe. However, the device may present potential hazards, particularly if it is operated by inadequately trained personnel or if it is not used in accordance with the intended purpose. Therefore, please read this manual for your own safety and protection. We have added numerous tips (written in italic) to help you.

GENERAL NOTES

Identification of the device and its certification

Device name: ClinoStar

Type, serial number: See the details on the back of the instrument

- **CE Certification**

This product complies with the European Union's Directive for radio equipment RED 2014/53/EU, EU Restriction of the use of certain Hazardous Substances (RoHS) 2011/65/EU and for low voltage electrical appliances 2014/35/EU 2002/96/EC and is marked accordingly.

- **WEEE**

This product complies with the European Union's Waste Electrical and Electronic Equipment (WEEE) Directive 2002/96/EC and is marked accordingly.

- **UL Approval**

This product has been certified by UL in compliance with relevant standards as described in the Declaration of Conformity.

This product is or may be covered by one or more pending patent applications (see www.celvivo.com/patents) for one or more countries including the US.

CELVIVO
STRESS-FREE 3D™

EU Declaration of Conformity
Supplier Declaration of Conformity

CelVivo ApS
Company registration number (cvr): 35671099
Middelfartvej 469
5491 Blommenslyst
Denmark

Hereby declare that the following product:

Identification:	ClinoStar
Model:	30002
Type:	01
Serial number:	30002-000001 ➡ 30002-999999

Is in conformity with the requirements of the following EC/EU Directives and Standards

Radio Equipment Directive 2014/53/EC & Low Voltage Directive 2015/35/EC
EN/IEC 61010-1:2010+AMD:2016 - General requirements
EN/IEC 61010-2-010: Particular requirements for laboratory equipment for the heating of materials
Corresponding UL-Standards: UL 61010-1:2010+AMD:2016 and UL 61010-2-010

EMC Directive 2014/30/EC
EN/IEC 61326-1:2013 – EMC requirements, Part 1: General requirements
EN/IEC 62311:2020 - Human exposure restrictions for electromagnetic fields
FCC 47, Part 15
ISED ICES-003
RSS-102

RoHS 2011/65/EC
EN/IEC50581:2012

2012/19/EU Waste electrical and electronic equipment (WEEE)

Date & Place: 15/12/2020 Blommenslyst, Denmark
Name & function: Peter Willems Alnøe - CEO

Peter Willems Alnøe

APPLICABILITY OF THE INSTRUCTIONS

Original document for translation

Keep these operating instructions close to the device so that safety instructions and important information are always accessible.

The content of the operating instructions are subject to change without further notice.

In case of conflicting translations into foreign languages the English-language version of these operating instructions shall be governing.

Should you encounter problems that are not described adequately in these operating instructions, please contact CelVivo Aps immediately (please visit https://celvivo.com/ for contact details).

WARRANTY

Requirements for warranty

CelVivo warrants the operational safety and functions of the ClinoStar only under the condition that:

- the device is operated and serviced exclusively in accordance with its intended purpose and as described in these operating instructions,
- the device is not modified,
- only original spare parts and accessories that have been approved by CelVivo ApS are used,
- inspections and maintenance are performed at the specified intervals.

Refer to CelVivo's general terms and conditions for more information on warranty and liability.

EXPLANATION OF SAFETY INFORMATION AND SYMBOLS

Safety information and symbols used in the operating instructions

	WARNING Indicates a hazardous situation which, if not avoided, could result in death or serious injuries.
	CAUTION Indicates a hazardous situation which, if not avoided, could result in minor or moderate injuries.
	CAUTION Indicates a situation which, if not avoided, could result in damage to property.

	Do not lift appliance
	Suffocation hazard
	Inhalation hazard
	Electrical shock hazard
	Unplug before proceeding

Symbols on the device

European Union CE mark

Waste Electrical and Electronic Equipment Directive 2012/19/EU

Equipment contains a UV-C light source. Turn off the UV lamp before servicing.

Refer to operating instructions to avoid hazardous situations

The operating instructions should be considered when operating the device

INTENDED PURPOSE OF THE DEVICE

CAUTION!

THIS EQUIPMENT MUST BE OPERATED AS DESCRIBED IN THIS MANUAL

If operational guidelines are not followed, equipment damage and personal injury can occur. Please read the entire Operating Manual before attempting to use this unit.

Do not use this equipment in a hazardous atmosphere or hazardous with materials for which the equipment was not designed.

CelVivo is not responsible for any damage to this equipment that may result from the use of an accessory not manufactured by CelVivo.

CAUTION!

LACK OF SAFETY DUE TO INCORRECT ACCESSORIES OR SPARE PARTS

Accessories and spare parts that are not recommended by CelVivo compromise the safety, function, and precision of the device. CelVivo cannot be held liable or accept any liability for damage resulting from the use of non-recommended accessories.

Only use accessories and original spare parts recommended by CelVivo.

Correct use

The ClinoStar is designed for preparing and cultivating cell cultures for research purposes only.

When operating, the ClinoStar provides a controlled environment conducive to cellular and multicellular growth by exerting precise control of the following parameters:

- Temperature
- CO_2 %

- Smooth rotation of the cell-culture vessel

This CO_2 clinostat-incubator has been designed to be installed and operated in the following areas:

- Laboratories for biological and biotechnological experiments of safety levels BSL1, BSL2 and BSL3 (Biosafety Levels set by National Agencies).
- Medical-microbiological laboratories in accordance with DIN EN 12128 (European Standard - Biotechnology - Laboratories for research, development and analysis - Containment levels of microbiology laboratories, areas of risk, localities and physical safety requirements).
- Research labs in universities, clinics, hospitals and other research Institutions.

This device is intended for professional use only and must **only** be operated by trained staff and cleaned by staff who have been instructed in the appropriate cleaning procedures (relevant to the work being carried out).

Incorrect use

The ClinoStar is not to be operated, maintained or cleaned by untrained staff.

The ClinoStar is not to be used for applications where materials are prepared for the introduction (e.g. medicines) or re-introduction into persons (regenerative medicine) or for *in vitro* diagnostics.

Do not use cell or tissue cultures in the device that are not in accordance with the regulations of safety levels BSL1, BSL2, and BSL3.

Do not use tissues, substances or liquids that:

- Are easily ignitable or explosive,
- Release vapours that form combustible or explosive mixtures when exposed to air
- Release poisons
- Release hazardous microorganisms or viruses that might be able to transverse a 0.22 µm pore size filter.

Do not use consumables or parts longer than recommend by CelVivo. This may jeopardize the safety of the persons operating the system.

Instruction of the operating personnel

We have prepared the ClinoStar to be as intuitive as possible and these instructions to help you get the most out of it, easily and safely. In this way, you can concentrate on your experiments and not have to concentrate on how to use this instrument. The ClinoStar has been manufactured in keeping with the latest technological developments and is operationally safe. However, the device may present potential hazards, particularly if it is operated by inadequately trained personnel or if it is not used in accordance with the intended purpose. Therefore, the following must be observed to prevent accidents:

1. The ClinoStar must be operated only by trained and authorized personnel
2. The user must comply with all operating instructions

3. Applicable safety data sheets, hygiene guidelines, and technical regulations (National, local, or institutional) which are in force in the users location.
4. The user must also instruct any cleaning personnel how to clean this instrument and issue personal safety instructions to any other persons who may come In contact with the instrument. Particular attention should be paid to:
   - which decontamination measures are to be applied for the device and accessories,
   - which protective measures apply when specific agents are used,
   - which measures are to be taken in the case of an accident.
5. Repairs to the device must be carried out only by trained and authorized expert personnel.
6. Any modification, deliberate or otherwise, of the Instrument will result in an annulment of the equipment warranty agreement.

Safety notes on gases

Note Installation work:

Any work on supply lines and pressurized gas containers, cylinders or containers used for storing CO_2, O_2 or N_2 must be carried out by expert personnel using the appropriate tools.

SUFFOCATION HAZARD!

LARGE AMOUNTS OF CARBON DIOXIDE (CO_2) RELEASED INTO THE ROOM ATMOSPHERE MAY CAUSE SUFFOCATION.

If a large amount of CO_2 is released, initiate safety measures immediately!
· Leave the room immediately and do not allow others to enter the room· Inform security service or fire department!

Note

It must be ensured that the CO_2 exposure limit at workplace is not exceeded. The country-specific national exposure limits at workplace must be met.

Safety notes on carbon dioxide (CO_2)

CO_2 is rated as a harmful gas. Therefore, certain safety instructions must be observed when the ClinoStar is installed and operated.

Instruction of personnel

Personnel operating devices requiring a CO_2 supply must be specifically instructed (including additional operating instructions of the gas supplier) in the correct handling of CO_2 before starting work including:

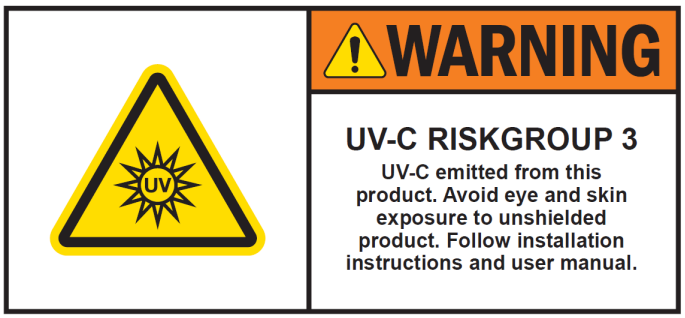
- correct operation of pressurized gas containers and gas supply systems,
- obligation to report damages and shortcomings in CO_2 supply lines,
- measures to be taken in case of accidents or failures.

These instructions must be refreshed or re-read at appropriate intervals.

Safety notes on UV-C

The ClinoStar contains a UV-LED emitting light at 275-280 nm. The lamp system in the ClinoStar is in Risk Class 3 assessed in accordance with EN62471-1.

UV-C radiation is potentially harmful and can cause damage to skin and eyes. The UV-LED is enclosed within the ClinoStar cabinet ensuring that light cannot escape the cabinet during operation. Therefore, to protect you from UV-C radiation, the UV-C decontamination routine can be run only if the front door is closed. During UV-C decontamination, the front door should remain closed. If the door is opened the UV-C lamp will shut down to prevent harmful UV-C radiation from being emitted from the incubation chamber.



Note on service work:

All service on the ClinoStar must be carried out by authorized service personnel. To avoid the risk of exposure to UV-C light, all service requiring access to the inner cabinet of the unit must be done without power on the device. If power is required for service, the door must remain open during service of the device.

INTRODUCTION

Thank you for selecting CelVivo products for your 3D cell culture needs. The CelVivo ClinoStar incubator is designed for use in industry, university, hospital and school laboratories or wherever there is a need to reliably culture cells in a clinostat in an accurately and reproducibly temperature and/or CO_2 controlled environment.

1. PRODUCT DESCRIPTION

The product consists of a clinostat-incubator unit, the 'ClinoStar' and is operated by a tablet.

The ClinoStar is an incubator specifically designed for long-term culture of multicellular structures (e.g. as biopsies, organoids or spheroids). It uses the clinostat principle to keep them in shear-stress free suspension. It is well-suited for long-term cultures and designed especially to minimize the risk of infections by unwanted microorganisms.

1.1. ClinoStar description

- The ClinoStar is a CO_2 incubator with 6 independent axles for the rotation of culture vessels (e.g. 10 mL ClinoReactors).
- Six cameras are installed in the door. These are used for monitoring the ClinoReactor in real time, with the images displayed individually on the tablet.
- A precision heating element and thermostat provides an accurate temperature control in the range from 6 °C to 20 °C above ambient.
- A CO_2 sensor provides a precise regulation of CO_2 levels from ambient to 10 %.
- Each axle has an associated motor which can turn a cell culture vessel (e.g. a ClinoReactor) smoothly clockwise or counter clockwise at speeds between 1,5 and 100 rpm (± 1%).
- A constant-speed fan placed in the centre of the chamber ensures a uniform internal environment throughout the chamber (and rapid recovery when the door has been closed).
- A UV-C light source can irradiate the whole of the internal chamber. The UV-C light is led through the fan shaft and directed out into all parts of the inner chamber, sweeping the surfaces like a lighthouse. The UV-C light can be activated when running a decontamination cycle. Decontamination cannot be performed while running an experiment. A safety lock ensures that the UV-C light is switched off if the door is opened and the decontamination cycle is aborted.
- All functions are conveniently controlled and monitored via a tablet. One tablet can control multiple ClinoStars. Multiple tablets can be used simultaneously on the same ClinoStar cluster.
- The unit has a small footprint and can be stacked up to 3 units high (using a stacking bar attached to the rear of the cabinet).
- The operator can open the door by a gentle push with an elbow. This will allow the user to have both hands free so as not to contaminate his or her hands and compromise the sterile environment.

1.2. ClinoStar Overview

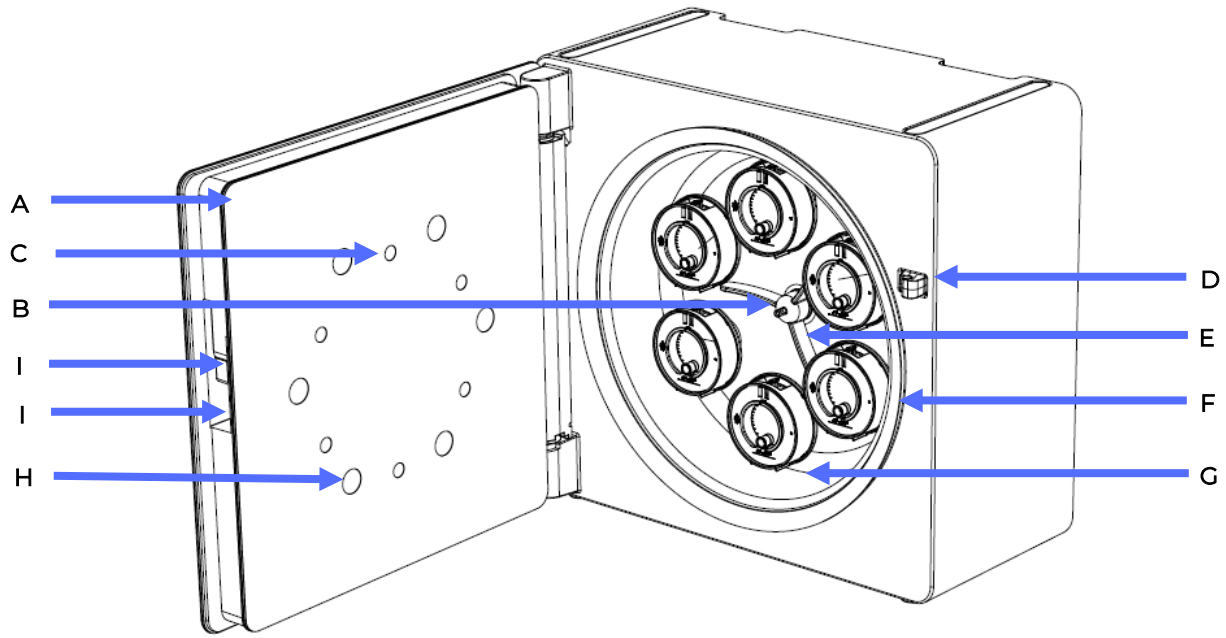


Figure 1

Principle components of the ClinoStar

A. Glass walled door
B. UV-C light source
C. Camera window
D. Door hatch
E. Fan
F. Door gasket
G. Stainless steel incubation chamber
H. Front LED light window
I. USB port (2)

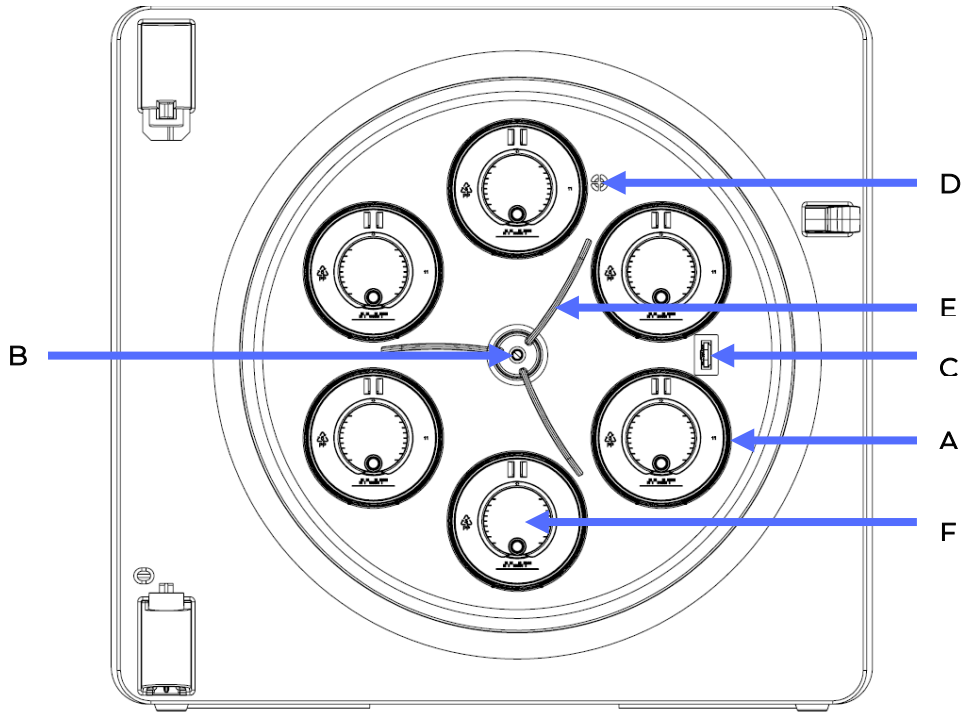


Figure 2

A. ClinoReactor and holder
B. Lighthouse UV-C light source
C. Temperature sensor
D. CO_2 sensor
E. Fan
F. Back LED light (in axle behind the ClinoReactor holder)

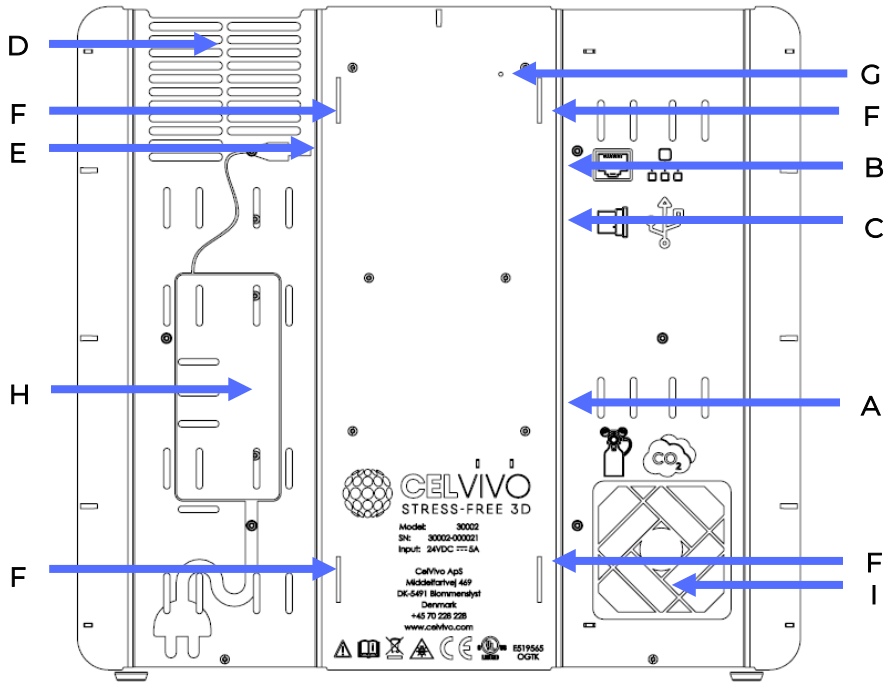


Figure 3

A. CO_2 connection
B. RJ45 internet cable connection
C. USB socket
D. Air vent
E. Power supply socket
F. Slots for stacking bars (used when you stack more than one ClinoStar)
G. Factory Reset (push with pin to reset)
H. Power supply bay (use Velcro straps to attach powersupply to device)
I. Cooling fan

1.3. Sensor system

Two sensors are mounted in the rear inner wall of the workspace:

- Sensor measuring the workspace internal atmosphere temperature (°C) ('C' in figure 2)
- Sensor for measuring the workspace internal atmosphere CO_2 content (%) ('D' in figure 2)

1.4. Fan system

The fan (E in figure 2) rapidly mixes the atmosphere inside the workspace. It runs at a constant speed when the ClinoStar is operating. *The fan can simply be removed by gently pulling it, when the fan is turned off, enabling an easier cleaning.*

The backside cooling fan (I in figure 3) is automatically controlled by the ClinoStar and will automatically switch on/off and adjust intensity. The controls are depending on the ClinoStar temperature set-point and the ambient conditions. Do not be alarmed if the fan is not running or running very slowly.

1.5. Door system

The door has a 'push-to-open' mechanism. Simply push the door gently in the middle right side (in the region indicated by the grey zone in figure 4). *Use your wrist, arm or elbow to avoid contaminating your gloves (or the ClinoStar).*

A gentle push is all that is needed. You will hear a click and after about 1 second, the door will open by about 90°. When the door is open, the UV-C light (if operating) is paused for safety reasons. The CO_2 valve shuts and the fan also stops.

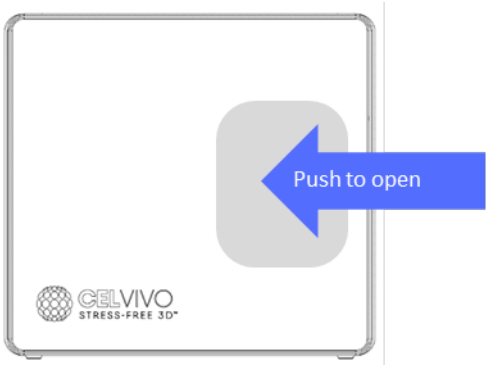


Figure 4

The door can be gently pushed shut again (*with your elbow*), upon which UV-C light and fan will restart and the CO_2 valve will open (if needed).

There is an indicator light in the top right corner of the door which provides information about the operational status of the unit.

Colour	Periodicity	Indication
Green	3 flashes, 10 sec pause	ClinoStar is ready to be set up
Blue	Continuous	Normal
Blue	1 sec. flashes	Normal – ClinoStar is selected on tablet
Yellow	0.5 sec flashes	Door open
Red	0.5 sec flashes	Alarm
Purple	0.5 sec flashes/ Continuous	Decontamination running (Selected/Not selected)
None		No power

1.6. Camera system

Six 5-megapixel cameras (C in figure 1) are installed in the door, in line with the axles. Each monitors a ClinoReactor and their video output can be seen on the tablet.

1.7. Lighting

The ClinoReactors can be illuminated either from behind (via LEDs placed behind them in the axles) or from the front (LEDs placed close to the cameras in the door). These lights are activated when the cameras are turned on and their intensity is automatically adjusted for optimal camera conditions. They can also be operated from the tablet.

1.8. UV-C decontamination system

3D cultures are often grown for months. Therefore, as one of the features designed to minimize the risk of microorganism contamination, the ClinoStar is equipped with a UV-C LED light (see 'B' in figure 2). This UV-C light is switched off automatically when the door is open. Do not activate the decontamination program while you have cells growing in the ClinoStar.

The UV-C light is emitted from the end of the fan, angled to ensure optimal exposure to the entire incubation chamber. As the fan turns, the UV-C light will thus sweep the workspace ensuring that the entire chamber is decontaminated.

1.9. Electrical data - Power supply

Electrical data	
Rated Voltage [V]	100 - 240 V
Power frequency [Hz]	50-60 Hz
Nominal power [A]	1.8A
Power cord length	2 meters
Appliance Class	Class I equipment
Pollution Degree	2
Overvoltage category	II

1.10. Electrical data - ClinoStar

Electrical data	
Rated Voltage [V]	24V DC
Nominal power [A]	5A
Pollution Degree	2
Overvoltage category	II

1.11. ClinoStar Specifications

Measurements	
External (d x w x h)	25 x 45 x 42 cm
External with door open (d x w x h)	64 x 45 x 42 cm
Weight	23 Kg
Internal diameter	305 mm
Internal depth	80 mm
Door	
Open mechanism	Push – click – swing open
Close mechanism	Push to close - click
Axles	
Capacity	6 axles
Speed range (rpm)	1.5 -100
Speed Accuracy	±1 %
Direction	Clockwise or counter clockwise
Control	Independent
Temperature data	
Temperature range	From 6 to 20 °C above ambient (capped at 41 °C) Please also see section on operation conditions
Temperature accuracy	± 0.25 °C
CO_2-data	
CO_2 range [Vol.-% CO_2]	Ambient to 10 %
CO_2 measurement	IR
CO_2 calibration	Factory calibrated for 10 years
Monitoring	
Cameras	6 (placed in line with 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 (A and A8) or 2000 x 1200 (A7)
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)

1.12. Tablet controller

The ClinoStar is controlled using the app preinstalled on the accompanying tablet (see the description starting in section 4.2.).

2. UNPACKING AND PLACING THE EQUIPMENT

CAUTION!

RISK OF PERSONAL INJURY!

The ClinoStar system including packaging weights approximately 30 kg and can be safely transported by a pallet lifter or similar.

When unpacked, the ClinoStar weighs approximately 23 kg. and should be moved by at least two persons.

The ClinoStar in its shipping box is approximately 56 cm (width) x 36 cm (depth) x 62 cm (height) and weighs approximately 30 kg. The shipping box is designed to be safely lifted with a pallet-lifter (figure 5).

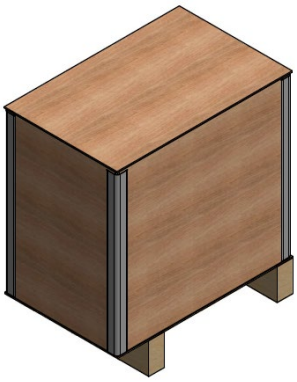


Figure 5

It should be shipped in the upright position, as indicated by the signs on the box and stored in a dry place.

Transport the ClinoStar in its original packaging only.

Move the chamber to the desired location and two persons should lift it from the rolling pallet.

Do NOT lift or transport the ClinoStar by holding the door.

Two persons should lift the ClinoStar, holding its four lower corners (and not the door).

It is recommended that the equipment is installed by a person certified to do so. Persons not certified should thoroughly familiarize themselves with this document before attempting the installation.

2.1. Arrival and unpacking

Upon arrival check the integrity of the shipping box.

In case of any damage, take some pictures and report it to CelVivo and to the shipping company immediately (without unpacking it).

If packing is undamaged and the shockwatch do not indicate impacts during transportation, unpack it, and check the unit and any optional accessories (see delivery receipt for list), for completeness and for absence of damage.

Please remove any transportation protection devices and adhesives in/on the chamber and on the doors and remove the operating manuals and accessory equipment.

Please wait until the unit has reached room temperature before installing. When brought in from a cold environment, it is recommended to leave the unpacked unit overnight to acclimatise. *Use the time to read the installation manual (this document).*

2.1.1. Content of package

The content of the package will depend on your order. Here is an illustration of possible included items. Specific items may differ from the illustrations

ClinoStar

Power supply and electrical cord

Manufacturer: FSP Technology, model: FSP120-AAAN3

CO_2 hose

Ø8 mm (o.d) 2 meter

CO_2 filter

User & service manual (this manual)

Accessories needed for installing more than one ClinoStar	
Nylon Y tube connector (for o.d. 8 mm tubing)	1
Stacking bars (for 2 ClinoStars)	1
Stacking bars (for 3 ClinoStars)	1

2.2. Installation requirements

The ClinoStar is designed for indoor use only. Requirements for the installation site for the ClinoStar are given below.

2.2.1. Choosing the site for installation

Choosing the site for installation of your new ClinoStar is very important. A good site will maximise its performance and minimise the risk of microbial infections.

- The room in which the ClinoStar will be used must be equipped with appropriate ventilation.
- The ClinoStar must **NOT** be placed directly under or in-line with the flow of air from any room ventilation, air conditioning unit or other equipment that produces an exhaust jet of air (e.g. certain centrifuges). (*Air jets are a major source of infections or contaminations and should be avoided).*

WARNING!

Installing the ClinoStar in an environment that does not adhere to the requirements set forth in this section might damage the unit.

The ambient temperature of the installation site must 19 °C or above to avoid condensation occurring inside the ClinoStar which can result in failure of electronical and/or mechanical components

- The location should be dry.
- To ensure a constant incubation temperature of 37 °C, the ambient temperature must be within the range of +19 °C to +30 °C.
- Do not place the device close to sources of heat (e.g. radiator or drying cabinet).
- The relative humidity should not exceed 70% (max.) and the conditions must not be condensing.
- The unit must not be exposed to direct sunlight.
- The minimal distance to adjacent surfaces which must be observed are given in figure 6

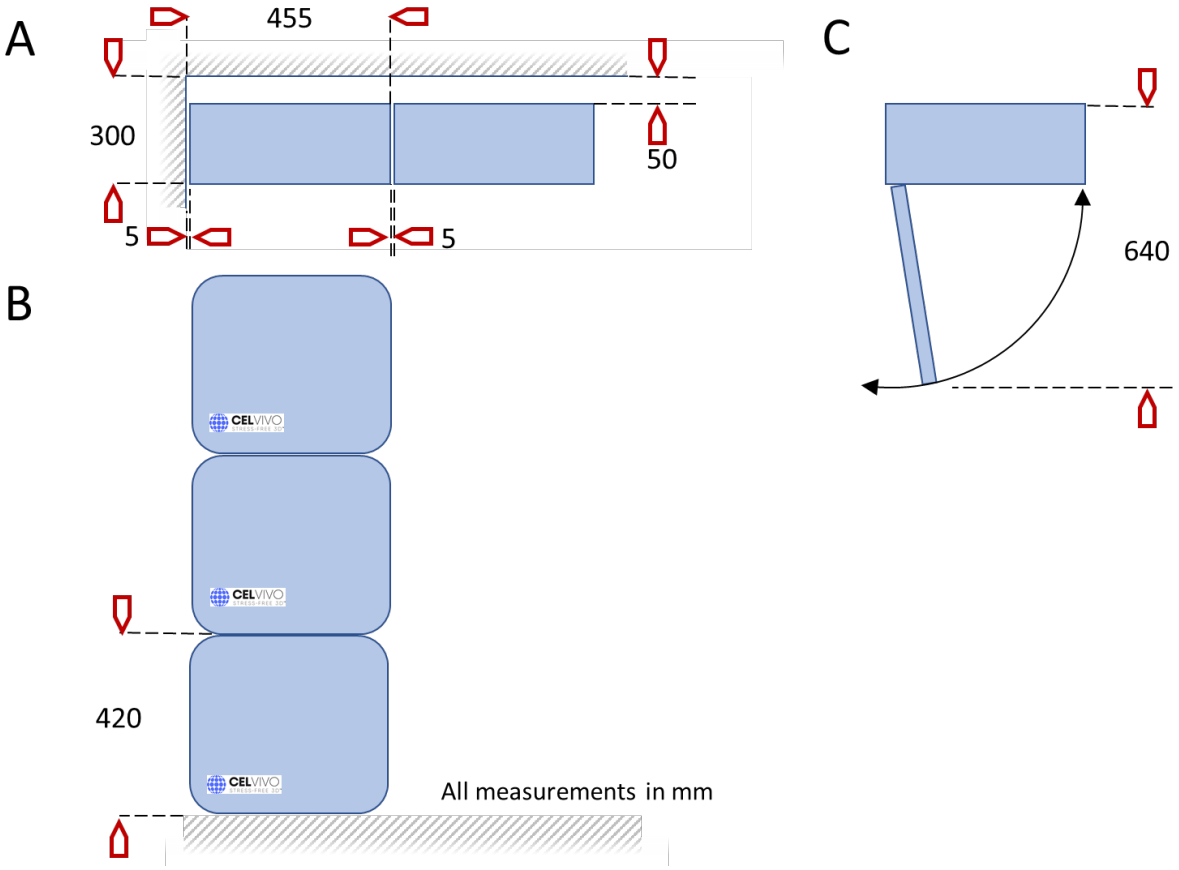


Figure 6

Minimal space around the ClinoStar is illustrated in figure 6. A) illustrates the unit from above showing clearance to side and back walls or other units, B) illustrates the unit from the front showing that it can be stacked 3 units high, C) illustrates the door opening arc. Shading indicates a wall, other equipment or the supporting surface. The space behind the units is required for the power cords and the CO_2 supply. The curved arrow indicates the space needed to fully open the door.

For optimal performance, the ClinoStar should be installed on a:

- Solid, level, fire-proof surface. It is important that the ClinoStar is level. If not the motion of spheroids or organoids within the culture chamber will be disturbed and can stress the cells.

- Vibration-free surface (e.g. a lab bench or wall-mounted shelf). (*Vibrations may affect culture growth).*
- The surface must be capable of bearing the total weight of the device(s) and accessories (take into consideration if you plan to stack several devices). *(We do not recommend placing the ClinoStar on or just above the floor since this will significantly increase the risk of infections).*
- The electrical and CO_2 sensing system of the device has been designed for an operating height of up to 2,000 m above sea level.

2.3. Special requirements for stacking

ClinoStars can be stacked on top of each other, up to a limit of three units. The bearing surface must be able to carry the combined weight of the ClinoStars and accessories (approximately 23 kg per unit). For safe stacking use the original CelVivo stacking stand) or the stacking bars and install as described in section 3. Contact your representative for more information if required.

2.4. CO_2 Requirements

For connecting CO_2 tanks to the ClinoStar we strongly recommend you use a two-stage CO_2 pressure regulator on the outlet valve of the gas cylinder (as is normally required for laboratory incubators). The input pressure must be maintained at 1 bar (≡15 ± 5 psi (pounds per square inch)) for the proper functioning of the CO_2 control system. *A single stage regulator will not stably maintain this pressure, resulting in inaccurate CO_2 levels*.

- CO_2 requirements quality: Purity 99.5 % min, medical gas quality
- CO_2 required operating pressure: 1 ± 0.3 bar (max. 2.0) bar
- CO_2 connection hose: Ø 4 and Ø 8 mm external diameter (supplied)

We recommend using medical grade gas that is at least 99.5% pure, because impurities in the gas can negatively affect your cells. The CO_2 gas supply should not have siphon tubes.

One connection will be needed for every ClinoStar.

2.5. Power connection

The power supply delivered with the ClinoStar (Manufacturer: FSP Technology, model: FSP120-AAAN3) can be used in USA/Canada, Europe and APAC utilizing the various power cords packed in the box.

Where extension cables have to be used, avoid plugging in other types of equipment that could lead to electrical noise or fluctuations in the power supply.

2.6. Network connection

The ClinoStar does not need a network connection for normal operation.

Individual ClinoStar units communicate using a wireless connection. For this reason, all units need to be within Wi-Fi distances. Typically, on the 2.4 GHz band they can reach up to 75 feet (23 m) indoors and on the 5 GHz about one third of this (although local building structures can significantly reduce both of these values). ClinoStar can utilize both bands but is set to use 5 GHz as default.

However, it does require a network connection for software updates.

The ClinoStar is equipped with a standard RJ45 connector on the back which must be plugged into an outlet providing the required IP-address, DNS and routing entries to allow for internet connection. Consult your IT-department before connecting the device.

The ClinoStar can also utilize available WIFI networks supporting either OPEN or WPA-PSK protected networks. This requires that the ClinoStar is extended with an approved and supported WIFI USB dongle. Refer to section 3.3.3 for more information.

We highly recommend that the ClinoStar is connected to the internet to ensure software updates of the Tablet and the ClinoStar. The devices and software can be updated without an internet connection to the ClinoStar. Contact CelVivo support if this type of update is required.

3. INSTALLATION

3.1. Installing the ClinoStar

If you are installing more than one ClinoStar please see section 3.2.

WARNING!

INCORRECT GAS INSTALLATION OR INSUFFICIENT VENTILATION IS DANGEROUS

Observe all National standards and regulations for the use/handling of gas. Installation and connection of gas tubing should be done by educated personnel.

WARNING!

RISK OF PERSONAL INJURY

Use a CO_2/O_2 alarm system for the lab if the room is not properly ventilated. Wear personal protective equipment (PPE). Check the tube connection system with a leakage test. During normal use, elevated levels of CO_2 may be found in and around the operating area of the ClinoStar.

Following these instructions sequentially will save you time.

1. Have a gas-leak detection spray ready or make a 10% SDS (sodium lauryl sulphate) solution.
2. Cut a piece of the 8 mm o.d. hose to a length suitable to reach the two-stage pressure regulator CO_2 outlet source or flask.
3. Connect the hose to the air filter unit available on the back of the ClinoStar.
4. Connect the hose to the two-stage CO_2 pressure regulator (which is in turn connected to the outlet valve of the gas supply outlet).
5. Control the pressure settings of the CO_2 inline pressure regulator. Note that the pressure unit may be shown in **bar** or **MPa** on the gauge of the inline pressure regulator. The output pressure should be 1 ± 0.3 bar or 15 ± 5 psi (pounds per square inch).
6. When the ClinoStar is not switched on, the gas valves are closed and it is therefore perfectly safe to perform a gas leakage test.
7. Pressurize the tubing by opening the pressure regulator to 1 ± 0.3 bar or 15 ± 5 psi and perform a gas leakage test (spray all the connections one by one and look for bubble generation). Proceed further only if there is no gas leakage from any of the tubing connections.

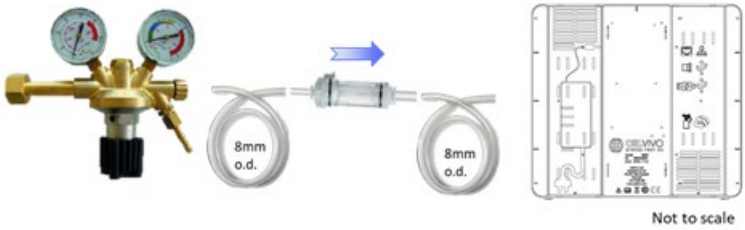


Figure 7

8. Confirm the voltage requirements available in your country are compatible with those required by the ClinoStar (see the label information on the back of the product). Select the appropriate power cord from those provided (do not use alternative cords).
9. Plug in the power supply into the back panel of the ClinoStar (see E in figure 3) keeping the ends of the power cord accessible. Strap the transformer to the back of the ClinoStar using the tabs provided.
10. If you wish to obtain internet connection through a wired connection, plug-in a standard RJ45 network cable into the correct socket (see B in figure 3) and plug the other end into the outlet.
11. Move the ClinoStar into its final position. Ensure that the gas hose and power cord are not trapped.
12. Plug in to the mains power and switch the power on.
13. The light on the front of the door at the top right corner will flash green to indicate that it is ready to connect to the tablet.
14. Leave your ClinoStar for an hour or so for the temperature and CO_2 levels to stabilise.

Your ClinoStar is now ready for use!

3.2. Installing more than one ClinoStar

1. Have a gas-leak detection spray ready or make a 10% SDS (sodium lauryl sulphate) solution.
2. Position the first ClinoStar close to its final position but at an angle that allows you to access the back panel.
3. Install the stacking bars, if the second unit should be placed on top of the first unit.
4. Position the second (and possibly third) ClinoStar beside the first unit (the third unit can be placed on top of the second).
5. Cut a piece of the 8 mm o.d. hose to a length suitable to reach the CO_2 outlet source (flask manifold or similar).
6. Connect the hose to the air filter unit available on the back of the ClinoStar.
7. Cut and attach pieces of the 8mm o.d. hose from the air filters to the CO_2 connector (manifold, Y-connector or similar).
8. Cut another piece of the 8 mm o.d. tubing to a length suitable to reach from the manifold or Y connector to the two-stage pressure regulator CO_2 outlet source or flask and connect. Allow sufficient extra tubing so that the ClinoStar can be moved for access to the back panel or for cleaning.
9. Open all the taps on the manifold.
10. Control the pressure settings of the CO_2 inline pressure regulator. Note that the pressure unit may be shown in **bar** or **MPa** on the gauge of the inline pressure regulator. The output pressure should be 1 ± 0.3 bar or 15 ± 5 psi (pounds per square inch).
11. When the ClinoStar is not switched on the gas valves are closed and it is therefore perfectly safe to perform a gas leakage test.
12. Pressurize the tubing by opening the pressure regulator to 1 ± 0.3 bar or 15 ± 5 psi and perform a gas leakage test (spray all the connections one by one and look for bubble generation). Proceed further only if there is no gas leakage from any of the tubing connections.
13. Confirm the voltage requirements available in your country are compatible with those required by the ClinoStar (see the label information on the back of the unit). Select the appropriate power cord from those provided (do not use alternative cords).
14. Plug in the transformers into the back panels of the ClinoStars (see E in figure 3) keeping the ends of the power cords accessible
15. If you wish to obtain internet connection through a wired connection, plug-in a standard RJ45 network cable into the correct socket (see B in figure 3) and plug the other end into the outlet. This step is only required for the first installed ClinoStar. If you connect more than one ClinoStar in a cluster they will share the internet connection of the master ClinoStar.
16. Move the ClinoStar which will have the lowest position into its final position. Ensure that the gas hose and power cord are not trapped under the second unit.
17. Lift one of the other two ClinoStars onto the hooks on the stacking bars and rest it on the lowest positioned unit (*a little help from a colleague makes this much easier and most*

importantly, safer). Ensure that the gas hose and power cord are not trapped under the second unit.

18. Repeat the above with the top ClinoStar.
19. Plug in to the mains power and switch the power on.
20. The light on the front of the door at the top right corner will flash green to indicate that it is ready to connect to the tablet.
21. Leave your ClinoStar(s) for an hour or so for the temperature and CO_2 levels to stabilise.

Your growing ClinoStar cluster is now ready for use!

3.3. Linking the ClinoStar and the controller app

Your ClinoStar is now connected to the CO_2 and power supply and ready to go. The front panel light should be flashing green.

Before operating the ClinoStar, it is necessary to establish a connection between the unit and the software app installed on the included tablet.

If you are setting up your first ClinoStar (or defining a new cluster of ClinoStars), proceed to the section 3.3.1 “Setting up the first ClinoStar”.

If you already have an operational ClinoStar and want to add an additional unit to your cluster, proceed to 4.3.3 “ClinoStar Setup”.

If you need to reconfigure your ClinoStar after initial setup, refer to the section 4.3.4. “Rearranging your ClinoStar cluster”.

3.3.1. Setting up your first ClinoStar

The first time you turn on a new ClinoStar software it will automatically start in configuration mode.

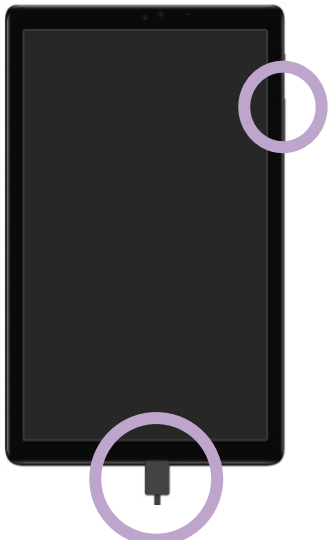


Figure 8

The power adapter must be plugged into the tablet as shown in the lower circle in figure 8 to charge the device. All tablet units have been preconfigured and charged before being shipped, however we recommend charging the tablet fully the first time you use it.

Please observe that the tablet can be charged directly from the ClinoStar. Simply disconnect the USB cable from the tablet power adapter and connect it to the ClinoStar using the USB charging port on the right side of the ClinoStar door (see ‘I’ in figure 1). Both ports can be used however we recommend using the port placed at the bottom.

Once connected press and hold the power button, marked with the upper circle, on the side of the tablet for a few seconds and wait for the device to start.

Figure 9

The tablet comes preconfigured from CelVivo. The first time the device is turned on it will automatically initialize. During this initial setup process, you *might* see the tablet setup screen (figure 9). No interaction is required.

If the ClinoStar is connected to the internet, you will see the same screen during update of the tablet and the ClinoStar. See section 3.3.3 on how to connect to the internet.

Figure 10

Once the App is ready, turn on the screen by pressing the power button (see upper circle in figure 8) once.

You should now see the lock-screen of the Tablet (figure 10).

Swipe up to start the program.

Figure 11

If this is the first time you turn on the Tablet, the application will automatically launch.

If you need to open the app manually, click the CelVivo logo at the bottom centre on the home screen as shown in the circle (figure 11).

Figure 12

When you start the App for the first time, you *might* be prompted to add the first ClinoStar if this has not been preconfigured. If you need to setup the ClinoStar this can be done following the description in section 4.3.3.

You need the ClinoStar configuration sheet which can be found in the top compartment of the ClinoStar shipping box (See figure 12)

If you follow the guide, the connection will automatically be established and the ClinoStar is ready to use. The control unit App will automatically sequentially assign a letter (A-Z) to each ClinoStar added to identify it in the App.

3.3.2. Setting temperature and CO_2 levels

Temperature and CO_2 levels and tolerances can be set individually for each of the ClinoStars in your cluster.

The default temperature is 37 ± 0.5 °C

The default CO_2 level is 5 ± 0.5 %

If any measurement falls outside of these tolerance zones, audible and visual alarms are activated.

If this is what you need, you're done!

If not, go to section 4.3.2 to set them manually.

3.3.3. Connecting to the Internet

To maximize the value of your ClinoStar we highly recommend that you connect the device to the internet. This ensures that you will receive future feature updates as they become available. Consult with your institutions IT policy before proceeding.

When the ClinoStar is connected to the internet, it will automatically ensure a network connection to the control tablet using the built-in access point. The access point is secured and only allows connection using the pre-set password (refer to the connection sheet packed with the ClinoStar)

Using a network cable:

To bring your ClinoStar online, simply plug-in the ClinoStar to the network using a standard network cabel (RJ45). The ClinoStar has no specific data routing requirements and will automatically obtain an IP (requires DHCP-server on your network). Should you or your IT-department have a question or need for support do not hesitate to reach out to CelVivo.

Using a WIFI USB Dongle:

CelVivo offers supported WIFI USB Dongles with the ClinoStar. Insert the USB dongle in the USB-port on the backside of the ClinoStar (C in figure 3). Please notice that CelVivo can not guarantee that all WIFI dongles will work. You should only use a dongle validated by CelVivo.

Use the “WIFI Setup” (1 in figure 13) below menu on the control tablet to configure your desired network profile. Once a network profile is saved, the ClinoStar will use that profile – also after the ClinoStar is rebooted.

Choose the WiFi you wish to connect to in the dropdown list (2 in figure 13) and the security type (3 in figure 13) and enter the password if applicable (4 in figure 13) and click connect.

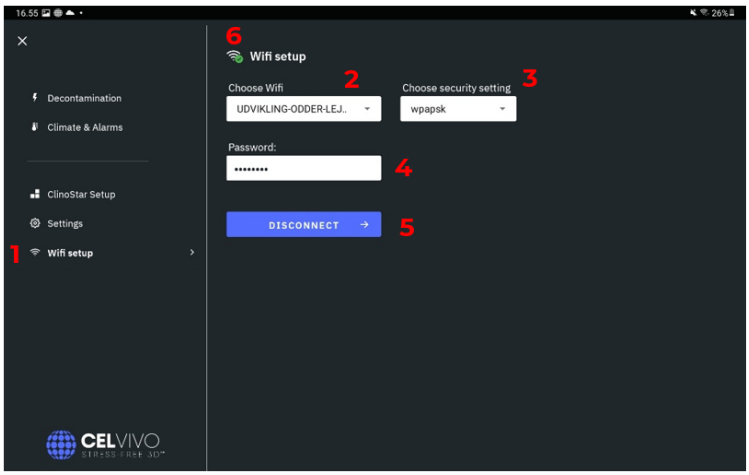


Figure 13

If you want to disconnect from the WiFi network, go to the WiFi Setup menu and click “Disconnect” (5 in figure 13)

Once a connection is disconnected a new WiFi profile can be defined. When a WiFi connection is established successfully, a green connection symbol will be shown in the WiFi Setup menu (6 in figure 13).

PLEASE NOTICE: If you have more than one ClinoStar configured in a cluster (refer to section 4.3.3), internet connection (either wired or through WIFI) should *only* be setup on the main Clinostar A. This will automatically ensure internet connection to the entire cluster.

3.3.4. After installation

After the ClinoStar is properly positioned, clean and disinfect the incubator interior and exterior (see section 5. Then, run a full decontamination cycle, as described in section 4.3.1. before running your initial culture.

4. OPERATION

The underlying concept behind the ClinoStar is that cell cultures should be disturbed as little as possible.

Thus, it is possible to inspect them without opening the ClinoStar. This avoids disturbing their growth environment while simultaneously removing a potential source of microorganism contamination. This means that you only need to interact with the ClinoStar unit when mounting or dismounting ClinoReactors .

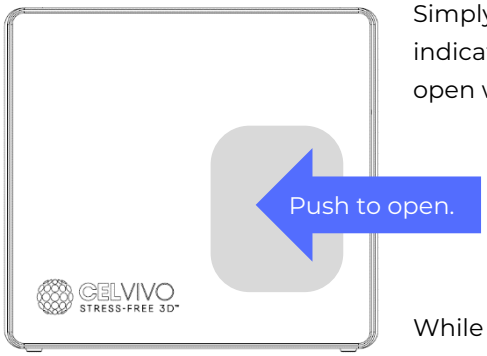
The ClinoStar is also designed to provide the optimal conditions for your research while minimising the chance of unwanted infections while being intuitive and easy to operate.

The ClinoStar is easily controlled by the app running on the accompanying tablet. One control unit can control between 1 and 50 ClinoStars.

If you are just setting up your first ClinoStar we would recommend that you check that your system has the latest version of the software and update if necessary. Please see sections 4.4.2 and 4.4.3.

4.1. Opening and closing the ClinoStar

3D cultures are often cultured for weeks, months or even years. Therefore, minimisation of potential infection by unwanted microorganisms is a key to success. To ensure as clean access to your cultures as possible, the ClinoStars is equipped with a 'push-to-open' mechanism.



Simply push the door gently in the middle right side (in the region indicated by the grey zone. After a delay of a few seconds the door will open wide (to about 90 °) allowing unhindered access.

While the door is open, the UV-C light will be switched off (if running), the CO_2 valve is shut and the fan will stop. All these functions will be resumed when the door is closed.

A gentle push is all that is needed to close the door. The flashing yellow light on the door will change to a steady blue when the door is properly closed.

To avoid potentially contaminating your gloves, you can use your elbow, wrist or back of your hand to push open or close the door.

4.2. Controlling the ClinoStar

Start the ClinoStars App by clicking the CelVivo icon on the home screen (as described in section 3.3.1.).

4.2.1. The App screen has five basic regions

The display seen on the tablet has 5 regions (see figure 14).

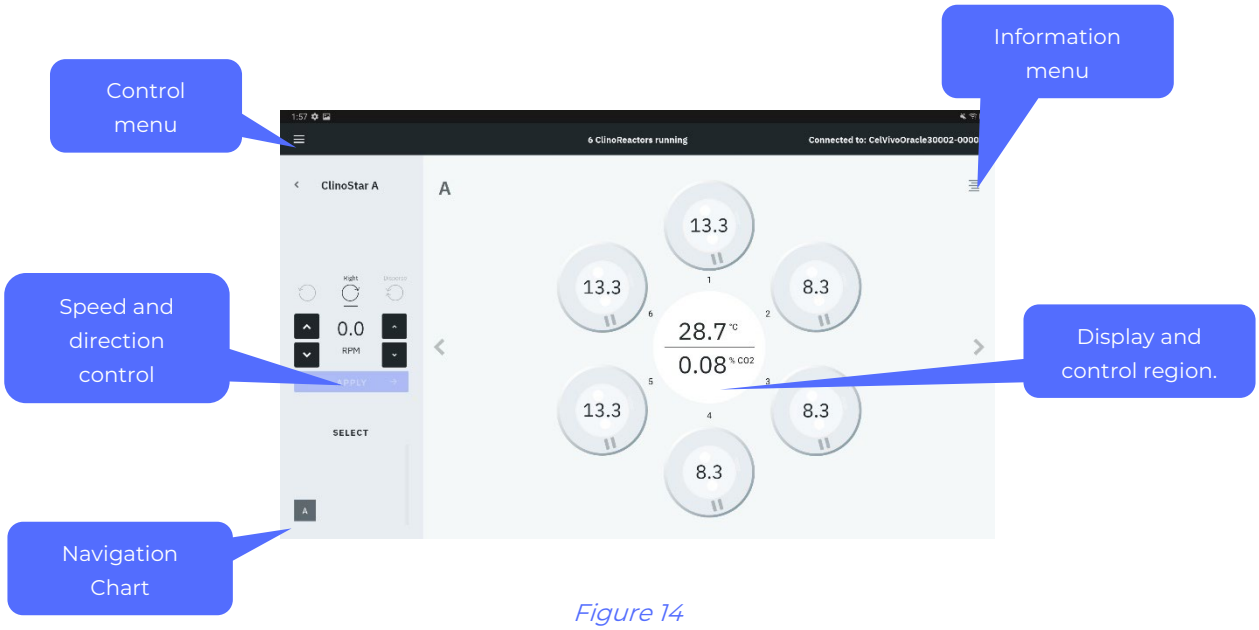


Figure 14

4.2.2. Navigating the App

The ClinoStar control App is structed around three levels, each giving the required overview and control at that particular level.

This symbol illustrates how to move between the levels or between ClinoStars

Level 1 displays a white pictogram for each ClinoStar in your cluster and any grouping that you have built (potentially matching the physical grouping in the lab) (figure 15).

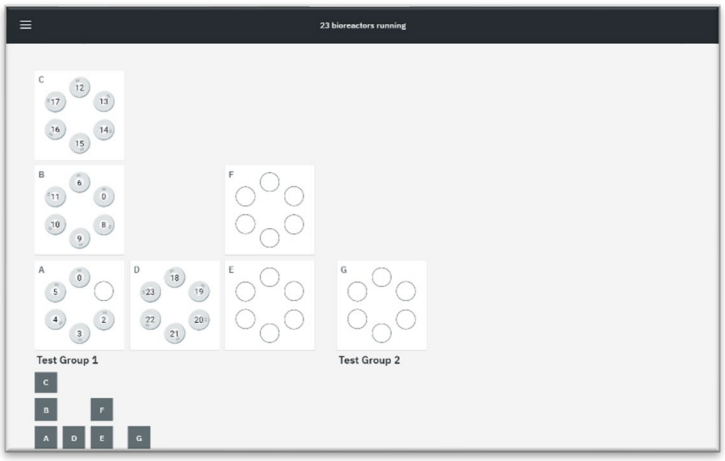


Figure 15

How to set this up is described in section 4.3.3.

Each white pictogram displays:

A unique letter (**A..B..C** etc. uniquely identifying each ClinoStar) in the top left corner)

6 circles (symbolising 6 axles). If a ClinoReactor is running, the circle will display the rpm and direction of rotation.

The ClinoReactors are also schematically shown in a navigation chart as grey squares with letters in the bottom left corner. If you are operating a large array of ClinoStars you can use the map to easily navigate left or right in the ClinoStar overview.

Touch any of the white pictograms to move to level 2 for that particular ClinoStar. The blue indicator light on the door of the selected ClinoStar will start to flash.

Level 2 displays the 6 axles in a particular ClinoStar (figure 16).

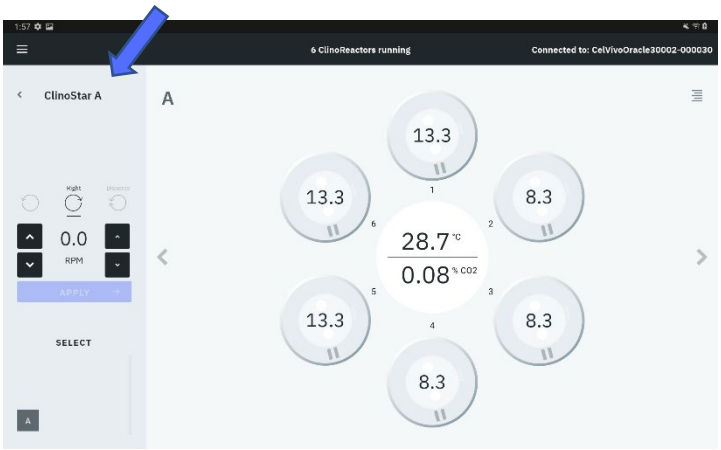


Figure 16

< **ClinoStar A** is shown in the top left corner.

Touching '**< ClinoStar A**' will lead back to level 1 and the ClinoStar will be deselected (the flashing blue light will stop flashing).

The speed control panel is below this.

The navigation chart is at the bottom.
You can quickly jump between ClinoStars by touching the appropriate grey pictogram

Axle symbols for each of the 6 ClinoReactors are shown in the display region. Each circle will display the rpm and direction of rotation.

The actual temperature and CO_2 (for this particular ClinoStar) are displayed in the centre of these 6 ClinoReactor symbols.

Swiping left or right will shift to the previous or next ClinoStar in your cluster (e.g. if you are viewing ClinoReactor B swipe to the right will lead to 'A' and to the left to 'C').

The Information Menu (≡ on the top right of the screen) will display the alarm settings for temperature and CO_2 (they are set in the control menu).

Touching any round **Axle** symbol leads to level 3 for that particular axle

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Level 3 displays the live feed image for a particular ClinoReactor (figure 17)

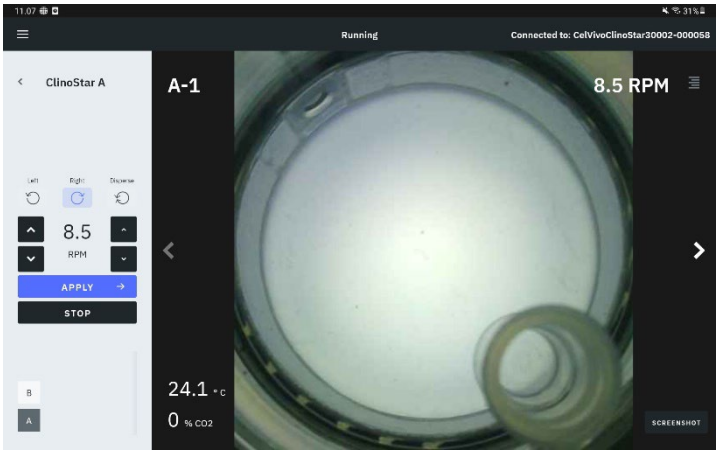


Figure 17

< ClinoStar A is still shown in the top left corner.

Touching '**< ClinoStar A**' will lead back to level 2.

The control menu button is still shown on the top left.
The left column still displays the speed controls and navigation chart.

The display area shows the live feed image for a particular ClinoReactor. The ClinoReactor is identified by its ClinoStar letter (A, B, C etc…) and position number (from 1 to 6 numbered clockwise from the top).

Top right shows the rotation speed.

Bottom left shows the current temperature (°C) and CO_2 (%).

Swiping left or right will shift to the previous or next ClinoReactor. Swiping left from 6 leads to 1 and swiping right from 1 leads to 6.

4.2.3. Level 1: the ClinoStar cluster overview screen

This screen illustrates all the axles in your ClinoStar cluster and their current status. You can see their rotation speed and direction.

If you have more ClinoStars than can be displayed on this screen, you can swipe left or right to see the rest.

This screen displays all alarms on all ClinoStars connected.

If there is any problem, you will see a flashing red centre of the ClinoStar and hear an acoustic alarm. The indicator light on the affected ClinoStar door will also flash red.

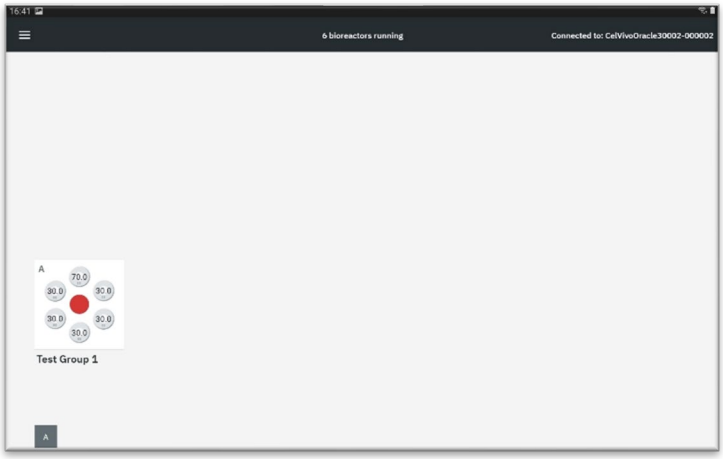
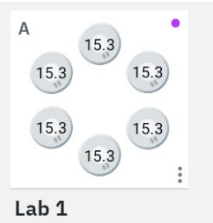


Figure 18

The acoustic alarm will cease if the problem has been corrected but can also be silenced by disabling the particular alarm.

The visual alarm, as shown in figure 18, will allow you to rapidly locate the problem(s). It will only disappear when the problem has been corrected. Click the specific ClinoStar to learn more about the alarm.

If an axle is not turning as programmed, the corresponding axle will turn red. Open the ClinoStar and see if something is blocking the axle. Contact your local CelVivo support representative if this is not the case or the error persists.



The overview screen also provides a fast overview of which ClinoStars are currently running a decontamination cycle using the small purple mark in the upper right (see figure 19). Refer to a later section on how to start the decontamination program.

Figure 19

Touch any of the white pictograms to move to level 2 for that particular ClinoStar.

4.2.4. Level 2: The ClinoStar detail screen

Clicking on **< ClinoStar A** will lead back to level 1.

Figure 20

The light grey circles on this screen illustrates all the axles in one particular ClinoStar and their current status (figure 20). Clicking on the right hand menu ≡ (1) shows the specific temperature and CO_2 settings and alarms for this particular ClinoStar. The actual temperature and CO_2 readings are shown in the middle of the main display (2).

Speed and direction of rotation of each axle are adjusted here using the main display (2) and the adjustment panel (3). ClinoReactors are numbered 1-6 clockwise.

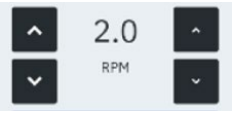
SELECT

To select axles, either click the **SELECT** button and select the axles you wish to control or **Touch** and hold one (or more) of the 6 axles until it (they) turn blue.

The direction of rotation can be selected by clicking the circular arrows in the adjustment panel.

The disperse function (double headed circular arrow) is used to rapidly resuspend your 3D structures after, for example, a medium change. If this toggle switch is on, the drive will reverse for 5 seconds before resuming the defined direction of rotation and turn again each time it has been stopped.

The speed of rotation can be adjusted by clicking the arrows, if a larger increase or decrease is required simply press and hold to fast-forward to your desired speed.



The two large arrows to the left changes the speed by 1 rpm per click. Holding the arrow is the same as repetitively clicking. The arrows on the right changes the speed by 0.1 rpm per click, allowing fine adjustment.

STOP Click to stop selected axles.

APPLY Click to apply your adjustments.

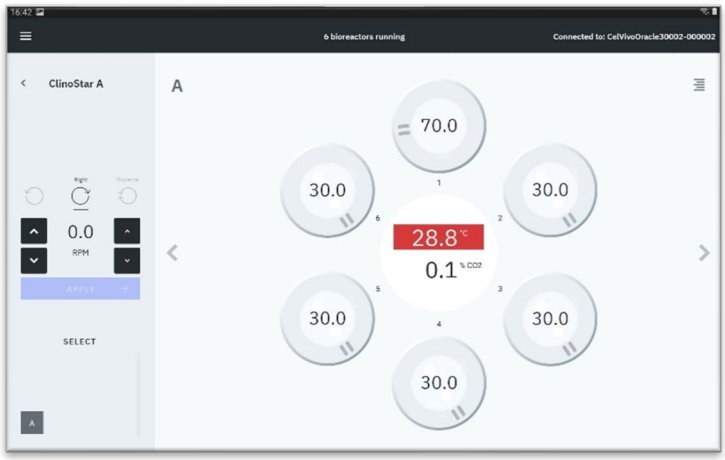


Figure 21

If an alarm level has been set on either temperature or CO_2 and the current state in the ClinoStar is outside these set levels, the background for the appropriate value (temperature or CO_2) will flash red as shown in figure 21.

If an axle is blocked or in any other way malfunctioning its pictogram will turn red. Open the ClinoStar and see if something is blocking the axle. Contact your local CelVivo support representative if this is not the case or the error persists.

Swiping left or right will shift to the previous or next ClinoReactor in your ClinoStar (e.g. if you are viewing ClinoReactor 2, swipe to the right will lead to '1' and to the left to '3').

Touching any Axle Symbol leads to level 3 for that particular axle.

4.2.5. Level 3: Live video from one ClinoReactor

Clicking on **< ClinoStar A** will lead back to level 2.

A key feature of the ClinoStar is to be able to see your culture in a live video feed from a specific ClinoReactor without opening the ClinoStar and disturbing the internal environment.

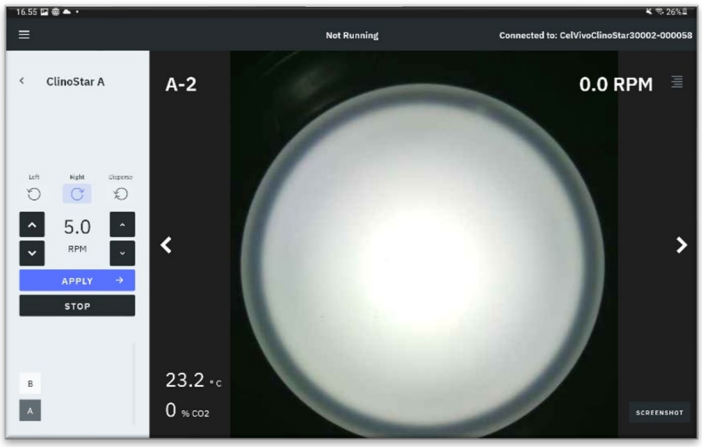


Figure 22

Lighting will automatically be switched on.

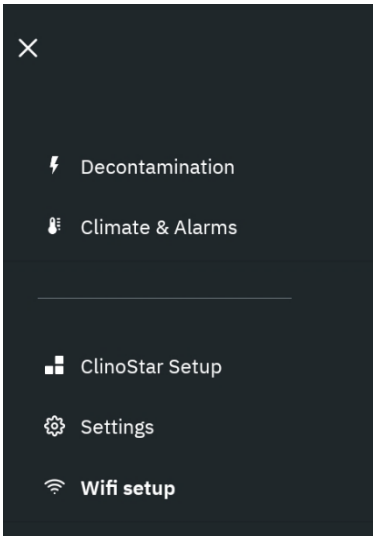
You can still adjust the speed using the left-hand side control panel (as described above) and see the alarm settings using the information menu (click the ≡ to toggle the information menu on the right in figure 22).

If you want to take a screenshot, to use in an article or similar, press the “Screenshot” button on the bottom right. Screenshots can be accessed by connecting the tablet to a PC/Mac using the USB-cable. Screenshot are stored in the Pictures folder in the Android filesystem (DCIM)

Swiping left or right will shift to the previous or next ClinoReactor. Swiping left from 6 leads to 1 and swiping right from 1 leads to 6.

Use the navigation chart at the bottom to quickly jump between ClinoStars.

4.3. Control menu



The left-hand side control menu is available at any time. You can access it by clicking the menu icon (≡) in the top left of the ClinoStar Control Application (figure 23).

In the control menu you set basic functionality (*that you should not need to adjust on a daily basis*).

These include controlling the decontamination program, setting climate and alarms, adding or removing ClinoStars and basic settings.

Figure 23

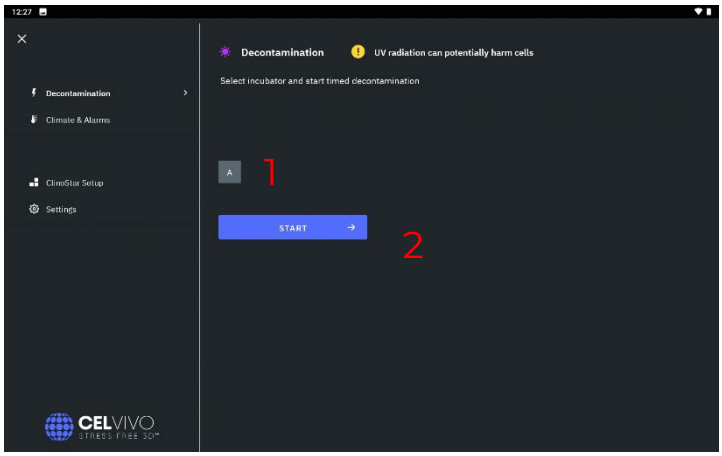
4.3.1. Running a decontamination program

To reduce the risk of infection, the ClinoStar has a build-in decontamination program using a UV-C light to sweep the internal incubation chamber. A full decontamination can be done in as little as two hours.

The decontamination program utilizes the internal UV-C beacon to ensure a uniform decontamination of all parts of the incubations chamber. During the decontamination cycle the ClinoReactor axles which do not have a ClinoReactor attached will slowly rotate to ensure a full coverage of the ClinoReactor holders.

***Warning**: Do not have ClinoReactors with cells in the ClinoStar while running decontamination.*

To engage the decontamination program, navigate to the “Decontamination” menu point in the left-and menu (refer to figure 22).



Select one or more ClinoStars to decontaminate (1) (figure 24). The selected ClinoStar will become grey in the selection field.

When ready to start the decontamination program click the “Start” button (2).

Figure 24

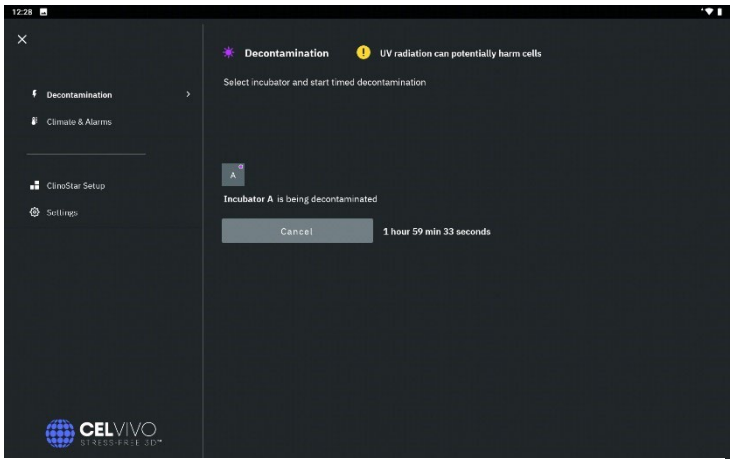


Figure 25

You can cancel the decontamination cycle at any time by clicking the “Cancel” button. Once engaged a timer will show how much time remains of the decontamination cycle (figure 25).

If the door is opened during decontamination the UV-C light will switch off and the program will suspend. Decontamination will resume once the door is closed and the user confirms on the tablet that decontamination should proceed.

hen the decontamination program is running, this will be shown on the level 2 controls screen with a large purple lightbulb above the temperature indicator as well as on the level 1 overview screen as described above.

Please notice: During decontamination, all alarms will be suspended. The fan as well as the ClinoReactor holders will rotate at a fixed pre-set speed. The speed, direction etc. of the engines cannot be adjusted during decontamination.

4.3.2. Controlling temperature and CO_2 levels

Temperature and CO_2 levels and tolerances can be set individually for each of the ClinoStars in your cluster.

The default temperature is 37.0

If this is what you need, you’re done!

If not, open the “Climate & Alarms” section in the right-hand side menu (refer to figure 23).

Figure 26

Choose the correct ClinoStar from the drop down menu that appears (figure 26).

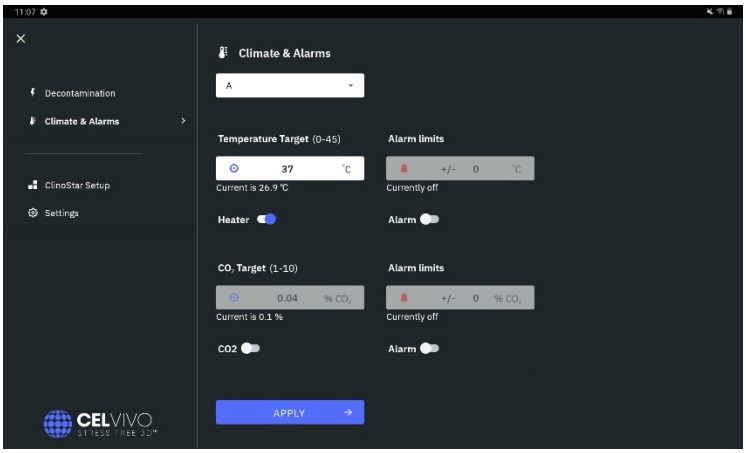


Figure 27

Enter the desired temperature and CO_2 levels by enabling temperature and/or CO2 respectively and enter the desired values in the temperature input field and CO_2 input field (figure 27).

Enter the desired tolerance for temperature and CO_2 and temperature and switch on the alarms as required.

APPLY

Click “Apply” to apply your changes.

When an alarm is triggered the App will display a warning on the first level screen and the LED on the appropriate ClinoStar door will flash red. Refer to the description of the overview and screens to see how alarms are displayed on the Control App.

To switch off an alarm level entirely, click the appropriate switch (denoted Alarm Limits). The alarm will be disabled until the alarm is rearmed.

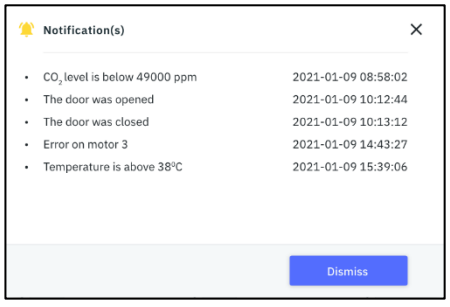


Figure 28

If an alarm occurred while the App was not active you will see the alarm log the next time you turn on the tablet – see figure 28. Based on the log, you can take an informed decision on how your experiments might have been impacted.

To reduce the risk of alarms being triggered before the ClinoStar has reached the desired operating conditions the ClinoStar is configured with the following grace period until alarms are armed:

- After system startup (powered on) alarms will not be armed until 45 min have passed to ensure that the required operating conditions (set point) have been reached.
- After the door have been opened a 10 min grace period will be observed before the alarms will be armed to ensure that the operating environment has been regained.

Please notice: The grace periods above will be voided if the operating conditions have been reached before the grace period has elapsed. I.e.. if the temperature and/or CO2 reaches the set points before the end of the grace period, the grace period will expire and alarms will be armed.

The tablet is used to show visual alarms and provide audio alarms, and therefore the tablet must be within WIFI-reach of the ClinoStar to relay alarm messages. An alarm will be triggered if the tablet loses connection to the ClinoStar.

4.3.3. ClinoStar Setup

One single tablet can control a cluster of up to 50 ClinoStars, giving your full control of your entire research environment from one central unit. Similarly, you can have several tablets running the same app to control the same ClinoStar cluster so that several users can work independently.

When you have added your first ClinoStar you can easily expand your cluster by using the menu item “ClinoStar Setup”.

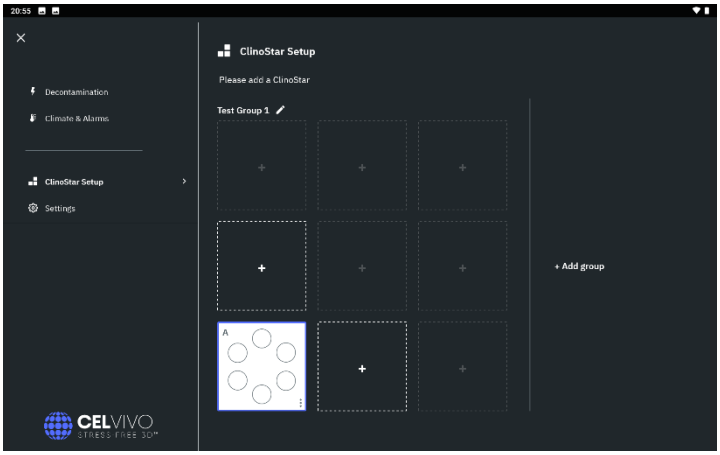


Figure 29

You can now add more by clicking the small + (plus-sign) either on top or to the side of your already installed ClinoStar(s) (figure 29).

This will allow you to virtually design your lab in the Tablet App to mimic your particular lab configuration for ease of use and overview.

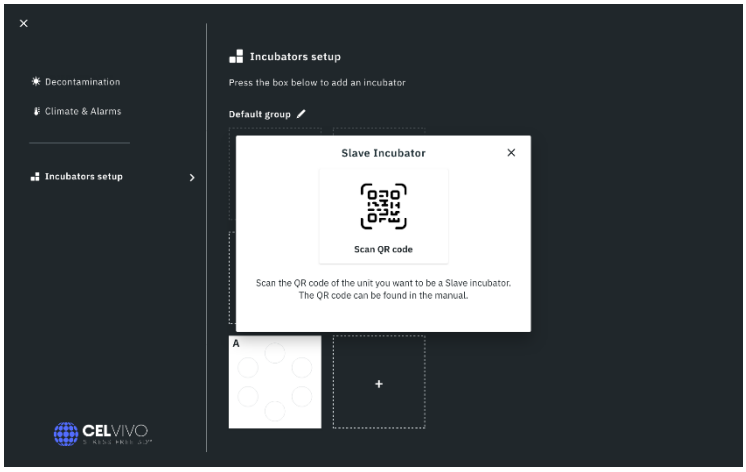
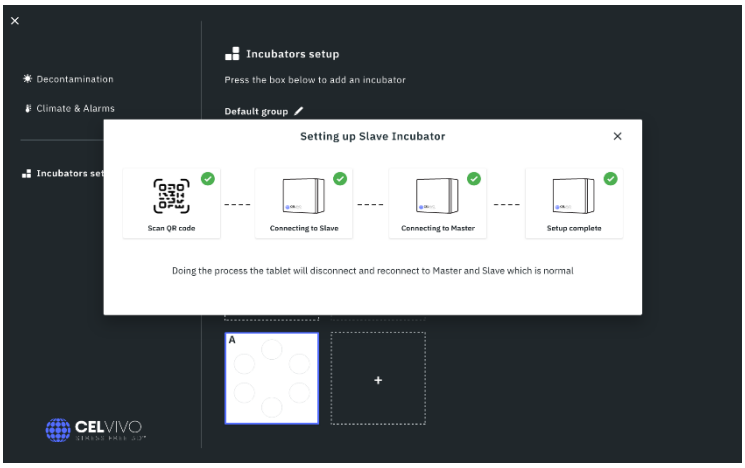


Figure 30

Once you clicked the + (plus-sign) you will be prompted (see figure 30) to scan the QR code of the ClinoStar you are adding to your cluster.

You can now scan the QR code (found in the manual or in the top compartment of the box you received your ClinoStar in) using the tablet camera.

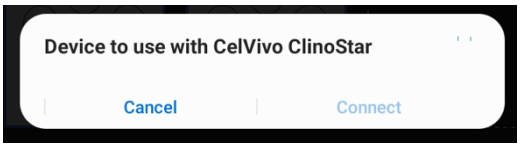
If you receive an error message that the ClinoStar you are trying to add cannot be found make sure its “ON” (power connected) and retry. If the problem persists, make sure you are within range of the ClinoStar WIFI (ideally in front of the unit you are trying to add).



The ClinoStars will now establish a connection to each other and exchange setup information. You can follow the progress on screen (figure 31). The process can take a few minutes to complete.

If you receive an error message that connection to the client could not be reached (step 2 in the process) click the “X” in the top right corner and restart the connection process.

Figure 31



During the setup process you *might* be prompted once or twice to verify which device to connect to during the setup process. Simply chose “Connect” in the dialogue box shown to the left.

You can also add a new section to your virtual lab layout in Tablet by clicking the “+Add group” to the far right (see figure 29). This will provide you with a new logical section in which you can arrange your ClinoStars.

To add clarity, you can name the defined layout groups by clicking the small pen to the right of the group name.

We recommend that you provide each group with an intuitive name that resembles the physical arrangement or use of the ClinoStars. *For example, one group could be ‘LEFT OF STERILE BENCH’ and another could be ‘BESIDE MICROSCOPE’. Alternatively, you could name the cluster according to the experiment or cell line that Is running in them, for example XYZ or C3A.*

4.3.4. Rearranging your ClinoStar cluster

After your initial configuration of ClinoStars, you can easily change the way Tablet shows the virtual layout of your ClinoStars for instance if you have changed your physical lab layout. We encourage you to keep this overview updated for ease of use.

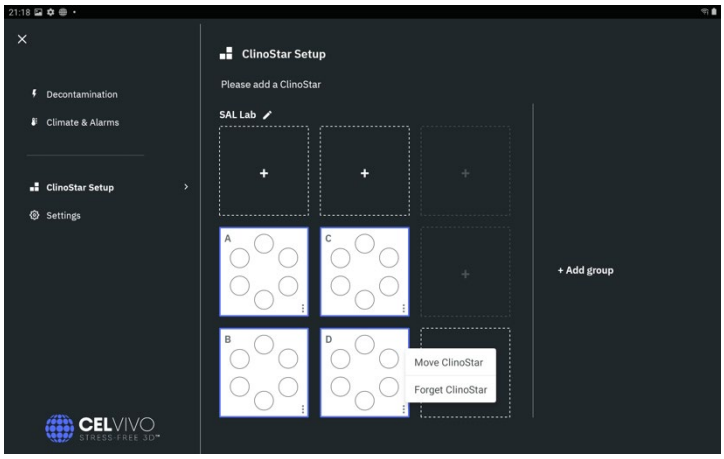


Figure 32

To change the location of a ClinoStar you use the “Move ClinoStar” option. Access the menu by clicking the menu button (≡) on the bottom right corner of the ClinoStar icon that you want to rearrange. Subsequently click the ClinoStar which you want to switch places / rearrange in the overview section (figure 32).

If you want to remove the ClinoStar fully from your setup click the button “Forget ClinoStar”. This should be done after powering down the specific ClinoStar you want to remove.

Warning: If you choose “Forget ClinoStar” you cannot re-add the ClinoStar to your cluster until you perform a factory reset and setup the ClinoStar as a completely new unit. If you want to perform a factory reset please refer to section 4.4.4 in this guide.

4.4. Settings

4.4.1. ClinoStar Software version

The tablet App software version is displayed in the section “App version” while the internal software version is shown under “Firmware version”. We recommend to always keep your ClinoStar environment updated with the latest software release. Refer to the sections 4.4.2 and 4.4.3 on how to update the software.

4.4.2. Updating the ClinoStar firmware

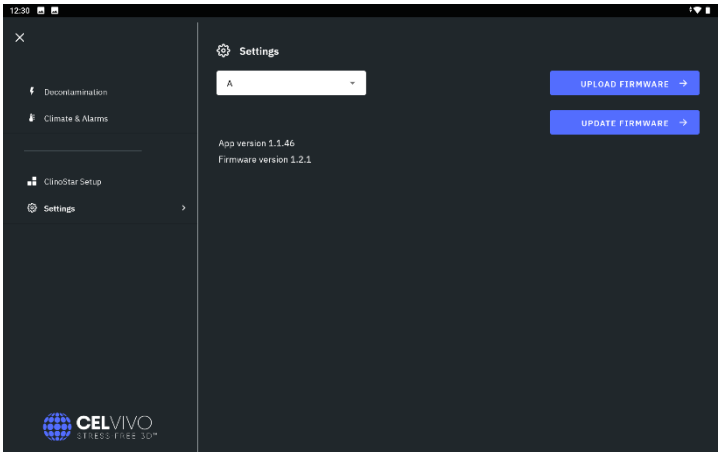


Figure 33

You can trigger an update of the internal software of the ClinoStar if a new release is available for a ClinoStar in your cluster. To ensure a high degree of integrity and safety for the experiments updating the ClinoStar firmware is a two-step process.

Select the ClinoStar you wish to update from the dropdown list.

Initially the new software package must be loaded to the ClinoStar. You initiate this process by clicking “Upload Firmware” (figure 33)

Loading the software will push the software package to the ClinoStar selected in the dropdown list but will not initiate the software update. This can be done without disturbing any experiments currently in progress. Once the software has been loaded to all the ClinoStar you can start the update process by clicking the “Update Firmware” button. The “Update Firmware” button will not become assessable before the “Upload Firmware” step has been performed.

CAUTION: *A software update should not be initiated while an experiment is running in the ClinoStar. We recommend that the software is loaded at once and that the update is triggered at a convenient time.*

Important*: Make sure that the door on ClinoStar is closed when initiating the update. The door must remain closed during the entire update process to ensure contact to relevant sensors. Do not open the door during the update process as this might interfere with the update process.*

4.4.3. Updating the control App software

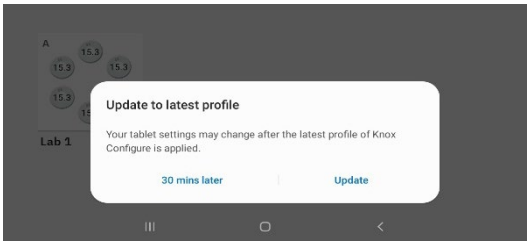


Figure 34

In the basic settings menu, you will also be able to see which version of the control unit app you are currently using. The control App is updated automatically and CelVivo will deploy new updates as they become available (see figure 34). Receiving updates requires that the tablet is connected to the internet, i.e.. through an internet connected ClinoStar or through a direct internet connection of the tablet.

You will be prompted to accept before any update is initiated. You can safely update the control App without impacting the experiments running in the ClinoStars and we always recommend deploying updates as early as possible to gain new features and potential fixes for errors and bugs. Once the update is complete the ClinoStar control App will automatically relaunch.

4.4.4. Resetting a ClinoStar or tablet

WARNING!

All data and settings will be lost and an initial setup needs to be performed.

Should you need to bring the ClinoStar back to factory settings, you can do so by pressing the small reset button located on the back side of the ClinoStar with a small pin (see ‘G’ in figure 3). This will trigger the

ClinoStar front-LED to flash red, green, blue. Open and close the front door two times while the LED flashes to initiate factory data reset. Be aware that **all data** on the ClinoStar will be **permanently and irrecoverably erased**.

Should you need to reset the tablet, perform a Factory Reset under “Settings” > “General Management” > “Reset” > “Factory Data Reset” and follow the on-screen instructions. Be aware that **all data** on the tablet will be **permanently and irrecoverably erased.**

If your ClinoStar is connected to the internet you can also contact CelVivo Support and ask them to initiate a remote reset of the ClinoStar or the tablet.

5. CLEANING AND DISINFECTION

Regular cleaning of the ClinoStar while sometimes a chore, is a necessity to help protect your cells from contamination and to keep the incubator functioning properly.

Carefully following these simple steps will keep your incubator clean, help reduce chances of contamination, and help keep your cells growing well. If you use the UV-C decontamination cycle that will kill bacteria and fungi, you should not need to use a disinfectant, but you should still periodically clean the incubator to eliminate any remains from spilled liquids, dust and dirt.

What you will need for the whole process:

- Protective gloves
- A mild dishwashing detergent solution (in two bowls)
- 10 lint-free cloths
- Distilled water
- Diluted quaternary ammonium disinfectant (follow the manufacturer's instructions for use)
- 70% Ethanol in water (in two bowls)

5.1. External

1. Clean the exterior of the ClinoStar to eliminate dirt and microorganisms that could find their way inside. If the top of your ClinoStar is dusty, it's possible that dust will pass into the chamber when you open the door, so it's important to clean the top as well. Ensure that dust is not blocking the backside cooling-fan (I in figure 3). If so, remove the dust gently using a small brush or vacuum cleaner on a low level effect setting.

   Note: if you find a lot of dust on the top, then you should consider if there is a better place to position the ClinoStar - perhaps further away from possible sources of dust like the ventilation system, air-conditioning or the door. If that is not possible, then you'll have to clean more often.

2. Use a lint-free cloth, soaked in mild soapy water and wrung almost dry to wipe all external surfaces. Pay special attention to the area of the door where everyone touches.
3. Repeat using a clean cloth which has been soaked in clear water and wrung dry.
4. Dry the outside with a clean, dry cloth.

5.2. Internal

1. If you have ClinoReactors in the ClinoStar, move all to a different ClinoStar.
2. Turn off the ClinoStar (this will close the internal CO_2 valve).
3. Remove the fan and door gasket. The fan can be removed by gently pulling. Remove the gasket by gently pulling it upwards on the top so it releases from the ClinoStar and thereafter gently take it off.

4. Clean all the internal surfaces, axles, inner door, fan and door gaskets with mild, warm and soapy water. Be sure to reach all the corners and crevices where dirt, dust and germs can hide. An incubator with curved 'corners' makes this task easier. Remember the back of the black ClinoReactor holders and the inner surface of the door and its gasket!
5. Rinse all surfaces and parts using a cloth wet with distilled water.
6. Wipe them dry using a clean, lint free cloth.
7. Wipe the interior surfaces and parts with a diluted quaternary ammonium disinfectant.
8. Follow this by wiping with 70% ethanol to remove any remaining traces of the disinfectant. Again, be sure to reach all the corners.
9. Repeat step 4 to 8 for the fan and the gasket.
10. Install the gasket and the fan again. The fan can be insert by gentle placing it back to its position, The gasket should be placed back starting from the top and gently placing the rim of the gasket in slit of the ClinoStar.
11. Now you can turn the ClinoStar back on and allow it to dry completely. This should only take a moment or two, so do not leave the door open – that would only reintroduce new dust and contaminants!
12. If you closed the CO_2 valve turn it back on (to 1 bar).
13. Now run the automated decontamination cycle (see section 4.3.1).

5.3. Tablet

1. Wipe the tablet with a lint-free cloth, soaked in 70% ethanol : water and wrung almost dry. It is a good idea to repeat this tablet cleaning procedure before starting to work and at least once a day

6. USER MAINTENANCE AND SERVICE

ELECTRICAL HAZARD

LETHAL RISK

The chamber must NOT become wet during operation or maintenance.

Do NOT remove the rear panel of the chamber.

Before conducting maintenance work, turn off the chamber at the main power switch and disconnect the power cable.

Any maintenance work must be conducted by licensed electricians or

The unit is designed to need a minimum of maintenance by the user. All other service should be carried out by a CelVivo approved technician. The user should regularly perform the following checks:

6.1. Cleaning

Perform according to use – but at least monthly, between experiments or after periods where the ClinoStar has been switched off.

See section 5.

6.2. Check the fan

Perform quarterly,

Check that the blades of the fan are not worn and cannot touch any of the receiving cups for the ClinoReactors.

6.3. Gas inlet filter

Perform yearly.

There is a filter in each CO_2 inlet line.

When the chamber is in operation, the incoming gas passes through this gas filter (aseptic filter, filtration efficiency 99.99 %, particle size 0.45 µm). This filter prevents dirt, which could be in the gas cylinder or in the supply hoses, accumulating in the gas inlet valves leading into the chamber.

When using gas with a technical grade of 99.5 %, we recommend changing the gas filter once a year.

When using gases with a lower purity, the filter should be changed more frequently. Please consult CelVivo.

6.4. Door sealing gasket

Perform yearly.

The door sealing gasket should be wiped with silicone once a year to keep it malleable. After a few minutes, wipe away any excess silicone.

7. SPARE PARTS AND ACCESSORIES

7.1. Spare parts

Description	Number	Product number
Fan	1	41323-286
Door Gasket	1	41323-221
Gas filter (on hose)	1	41900-001
Hose	1	41900-002
RJ45 network cable (3 meters)	1	41900-003
Wireless Dongle	1	41902-004

8. DISPOSAL

8.1. Disposal of the transport packing

Packing element	Material	How to Dispose
Door protection	PE foam	Plastic recycling
Packing box	Wood	Wood recycling
Insulating air cushion foil	PE foil	Plastic recycling
Bag	PE foil	Plastic recycling

If recycling is not possible, all packing parts can also be disposed of with normal waste

CAUTION

CONTAMINATION HAZARD!

As the device can have been used for processing and treating infectious substances or human cells, it may be contaminated. Prior to disposal, the complete unit, with filters and hoses, must be decontaminated by performing a formaldehyde sterilization.

8.2. Disposal of a ClinoStar

This product complies with the European Union's Waste Electrical and Electronic Equipment (WEEE) Directive 2002/96/EC and should be disposed of accordingly.

The ClinoStar contain reusable materials and should be disposed of accordingly. All components, with the exception of the HEPA filters, can be disposed of after having been thoroughly cleaned and disinfected. All parts, including HEPA filters and the tablet, must be disposed of in accordance with the applicable National, state and local regulations.

8.3. Recyclable materials

Component	Material	How to Dispose
Thermal insulation components	Polystyrene foam, EPS/PPS compound	Plastic recycling
Printed circuit boards	Enclosed electrical components coated with various plastics, mounted on epoxy resin-bound boards	Electrical recycling
Plastic components, general	Note material labelling	Plastic recycling
Exterior housing	Steel, painted	Steel recycling
Device back panel	Stainless steel	Steel recycling
Front door	Multi-layer safety glass	Glass recycling
Tablet batteries	Lithium ion	Batteries

9. How to get help

There are several ways to get help. Please choose the most appropriate way for your needs.

For General Guidance:

User login at **CelVivo.com**. Have a look at this restricted region of the website where we will post a growing number of useful SOPs, tips and hints from other users.

For Technical Help: Contact your local supplier (their number is on CelVivo's website). If they cannot solve your problem, they will immediately contact CelVivo ApS, Ørbækvej 264, 5220 Odense, Denmark, info@celvivo.com, phone: +45 70 228 228.

For Scientific Help

Guidance as to how to start or plan experiments, discuss unusual observations or difficult cultures:

Contact CelVivo directly at info@celvivo.com or +45 70 228 228.

For Inspiration:

Publications generated using the ClinoStar will be posted on the open region of CelVivo's website.

For Anything Else:

Contact CelVivo directly at info@celvivo.com or +45 70 228 228.

10. Frequently asked Questions

As a rule of thumb, when you start to grow cells in 3D in the ClinoReactor we would recommend that you continue to use the same conditions for the cells as they are used to (media, additives, temperature and CO_2%). This usually works. Once you have a successful set of conditions, it is possible to vary some of the parameters to optimize the cultures.

One important feature to remember is that cultures typically consume the glucose (and possibly other nutrients and growth factors) much more rapidly than seen in 2D cultures. This is usually due to the fact that there are more cells per mL than in typical 2D cultures. Therefore, keep the number of spheroids or organoids down until you are confident in producing 3D cultures.

Q: Did you ever experience aggregate formation during cultivation? What do you recommend to get rid of, or avoid aggregates?

A: Formation of irregular big aggregates during clinostat bioreactor cultures can be related to several factors:

- the cell/spheroid population is too dense in relation to available volume.

- cell conglomerates (spheroids, organoids, or biopsies) are not uniform.

- “condensation centres” such as bigger agglomerates, fibres or contaminants are present in the vessel.

- highly proliferative cells with strong migration and attachment properties are being used (e.g. cancer cell lines with high metastatic potential) for creation of clinostat cultures.

We would recommend following general practice to avoid unintentional agglomeration of spheroid cultures:

- use a less dense starting population: This will give more space for individual spheroids and will make it easier to find the optimal rotation speed.

- at any given time point the vessel can only have one rotation speed and therefore having a uniformly sized spheroid population is crucial. This is especially important during the initial phase of clinostat culture (usually the first week for immortal cell lines).

- if the growing population tends to have different sizes, we recommend separating them into different ClinoReactors (by manually sorting into different sizes in different vessels).

- uneven spheroid sizes or any other odd size objects will create agglomeration centres (especially during initial phase of culturing – first week) therefore it is important to remove them as soon as they are seen.

- in some cases, when highly proliferating cells (e.g. some highly malignant cell lines) are used for spheroid cultures, we would recommend coating the initial spheroids with degradable materials (e.g. we use sodium alginate coating (see the appropriate SOP) to prevent their agglomeration).

Q: Have you ever experienced that spheroids quickly adhere to the ClinoReactor surface e.g. during gardening or image acquisition? Would you say this is cell type specific?

A: In general, the materials used for our ClinoReactor are very low attachment materials. If cells/spheroids are attaching to the ClinoReactor plastic surface, we would speculate that some kind of protein coating has been deposited on the plastic. It could be secreted protein or protein released from damaged cells. Try moving the culture to a fresh ClinoReactor and change them more frequently.

Q: It seems that the aggregates accumulate in the lower left area of the bioreactor (or right depending on the direction of rotation). Neither increasing nor decreasing the rotation speed results in improvement. How can the rotation speed be adjusted if aggregates are formed?

A: Aggregation of big conglomerates in one spot inside clinostat bioreactor is a result of balance between rotation speed/viscosity of the liquid/ surface of the conglomerate/weight of the conglomerate = lift capacity. The placement of steady spot on left or right side depends on direction of the clinostat rotation.

When big aggregates are already formed most usually changing of the rotation speed will not result in dispersion of the aggregate. Either discard the aggregates or select good spheroids and move them to a new ClinoReactor.

Q: How often do you use the “disperse mode“ (cw/ccw) during establishment of spheroids from unknown cell types?

A: The “Disperse mode” is used to facilitate fast and even redistribution of conglomerate population inside the clinostat bioreactor vessel volume. I would advise to use it after every time the ClinoReactor rotation has been stopped (e.g. for bioreactor handling), or the speed of rotation has been changed by more than 5%.

Q: Which preparation method do you recommend for cells generated from tissue biopsies? Could the preparation method lead to aggregate formation? Are there too many spheroids in the bioreactor?

A: The preferred aggregation method will very much depend on properties of cell type(s) used. In general, for single cells generated from tissue biopsies (cells with relative high oxygen demands and already coming from 3D structures) we would recommend methods which allow quick transfer to the ClinoReactor (e.g. ‘force’-aggregation with use of scaffold/hydrogel materials like sodium alginate) or the direct self-aggregation of single cell suspension in clinostat bioreactor vessel.

In general, try to use less dense population for starting the clinostat cultures from this type of cell. A too dense population is one of most often causes of spheroid conglomeration

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Q: Is it okay to use fungicides like amphotericin B during the cultivation?

A: Considering the materials used to make the ClinoReactor there is no problem to use the amphotericin B during spheroid culture.

If we consider the spheroid population itself, the answer depends on what type of research the spheroids will be used for.

Depending on the type of research, as well as lab conditions and routines, the use of certain media additives can be permitted or needed.

In our practice, we successfully have culture spheroid population without any additives (antibiotics or fungicides) for extended periods of time (up to a year). For ease of use and for experiments where it is not expected to affect the results, we use an antibiotic cocktail in our standard growth media (Pen/Strep 0.5% v/v).

Q: Which strategy would you recommend for drug treatments/screenings with biopsy samples (high spheroid number and aggregates; not countable)? In such case, one biopsy sample is cultivated in one bioreactor and treated as a whole. How can you make sure that the amount of spheroids is comparable between samples? Are there any non-invasive methods to use as normalization parameter for this kind of study design (protein/ATP/area in normal spheroids)

A: If you would like to use the big single spheroid conglomerates for your experiments, we would suggest following normalization methods:

- non-invasive volume calculation based on conglomerate photography.
- if the conglomerates are bigger than 3-4mm in diameter we have successfully used wet mass weight to normalize the treatment experiments.
- if the treatment is relatively short and terminating you can use known part of sample to perform the biomass calculation by protein determination.

If you have further questions or tips that you have, we would love to hear them. We will pass them on to all ClinoStar users and hopefully save them time and effort.

Version 2.01