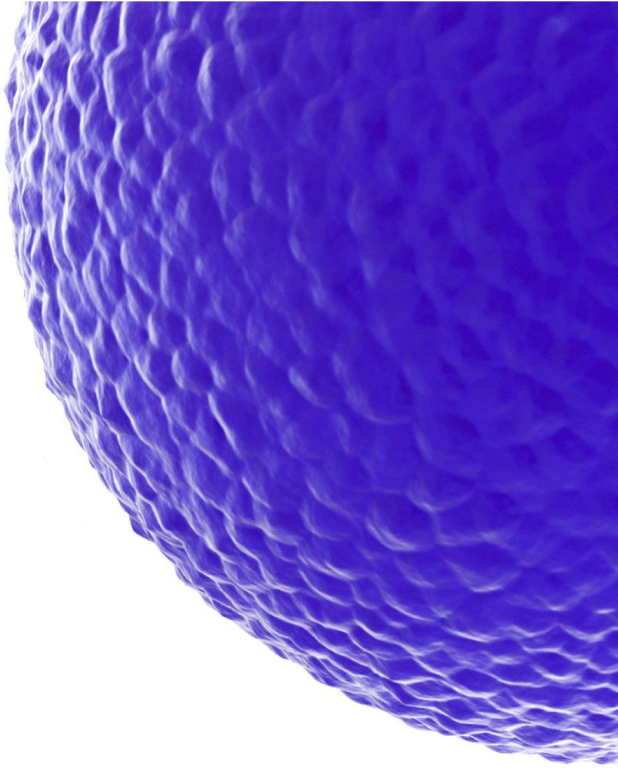

CELVIVO

STRESS-FREE 3D™

ClinoReactor

Product Introduction

Rotation of the ClinoReactor® keeps cells and 3D structures in shear stress-free suspension, promoting a high degree of cell-cell interactions which in turn leads to the maintenance (or recovery) of tissue like functions.

The ClinoReactor® provides a rapid gas exchange between the growth medium and the surrounding atmosphere. This provides oxygen for the cells and CO_2 to balance the pH (for bicarbonate-buffered media).

As the ClinoReactor® turns, the shear force between the vessel and the growth medium causes the growth medium to turn with the vessel. The actual shear force experienced by the growth medium will depend on its viscosity.

3D structures in the growth medium will thus be acted upon by viscosity (as the medium flows past the structure) and gravity.

Once the uplift due to movement of the growth medium (relative to the 3D structures) matches the down-pull of gravity, the 3D structures will be kept in an essentially shear-stress free suspension and will appear to be in a stationary orbit around the axis of rotation. Removal of shear stress is a key factor to allow the cells to attach to each other. There will be a residual flow of medium past the 3D structures and this is key to reducing the diffusion depletion zone.

Now, because gravity will act more strongly on larger structures than on smaller ones, because they displace a larger volume, a higher rotational speed is needed to keep larger structures in suspension. In a mixed size population, the rotation speed may be OK for the small structures, but larger ones may sink to the bottom. Differences in size will result in some structures experiencing increased shear forces. Therefore, it is highly advantageous if all the 3D structures are the same size and shape because they will all tend to remain in a shear stress-free stationary orbit.

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.

Product Description

The ClinoReactor® has a reaction volume of 10 mL, surrounded by a humidification chamber containing humidification beads which allow the absorption of 25 mL of sterile water.

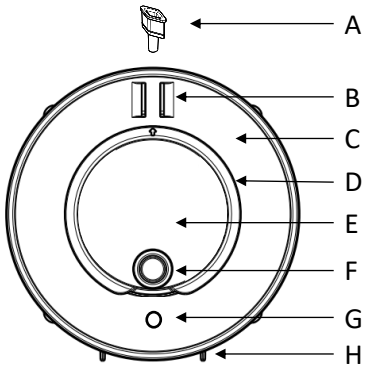

Figure 1 ClinoReactor® for single use. (A) Top plug enables media dispensing and removal. **(B) Vents** to ensure correct gas exchange and humidification in the culture chamber. **(C) Humidification chamber** containing the unhydrated humidification beads. **(D) Petri dish lid** for opening the entire culture chamber in a petri dish fashion. **(E) Cell culture chamber**. **(F) Front port** giving access to the culture chamber. **(G) Hydration port** for hydration of the humidification beads with sterile water. **(H) Feet** allowing the ClinoReactor® to stand upright.

The two air vents (**B**) allow rapid exchange of the external atmosphere with the internal atmosphere, keeping the CO_2 and temperature levels the same as in the ClinoStar®.

Humidification beads (**C)** slowly release water and maintain a very high humidity in the internal atmosphere, allowing efficient gas exchange across the internal membrane.

The culture media can be added, exchanged or removed through the top port after removal of the top plug (**A**).

The culture chamber (**E**) can be opened to provide petri-dish like access to individual organoids or spheroids by removing the lid (**D**). When closing the petri dish lid, it 'clicks' twice when shut. For less exposure, the front port (**F**) could also be used to collect 3D structures.

The unhydrated humidification beads is hydrated by injection of 25 mL sterile water into the hydration port (**G**).

The feet (**H**) stop the ClinoReactor® from rolling while you are changing media.

Storage and Stability

Store ClinoReactor® vessels at room temperature (15 - 25°C) away from direct sunlight. Stable for 1 year from date of manufacture (MFG) on label.

Product Information

Product Name	Catalog #	Size	Description
ClinoReactor®	10004-12	12 x Bioreactors 12 x 25 mL WFI water ampoules 1 Lifter tool 1 Contrast plate 4 x spare top plugs (Sterile)	ClinoReactors with a 10 mL reaction chamber 25 mL WFI water ampoules for hydration of blue humidification beads Make the opening of petri dish lid easy For having a colour contrast when studying cells in the ClinoReactors outside the ClinoStar®.

Directions for Use

This protocol describes how to prepare a ClinoReactor® vessel for use in experiments. ClinoReactor® is delivered double wrapped and should be handled in a sterile environment such as a laminar-flow biosafety cabinet.

Reagents and Materials

- ClinoStar®
- ClinoReactor®
- 25 mL Sterile water (supplied)
- Sterile water for washing (Note: PBS or HBSS could also be used)
- Cell culture media

Additional information

As the cell cultures are maintained for a prolonged period, it is exceedingly important to work as sterile as possible to minimize the risk of infections. When equilibrating the ClinoReactor® and filling it with 6-8 mL of media, the aircushion will assist in removing air bubbles that could be in the chamber.

Protocol

Hydration of the humidification beads

1. Open the outer package and transfer ClinoReactor® to the sterile workspace.
2. Collect the hydration beads at the bottom of the ClinoReactor®.
3. Place the ClinoReactor® on a flat surface with the front facing upward.
4. Hydrate the humidification beads by adding 25 mL sterile water through the hydration port by piercing the inner package (**Figure 1 G**).
5. Keep the ClinoReactor® with the front facing upwards. Wait minimum **4 hours** until the

humidification beads have absorbed the water.

6. Optional: After hydration of the beads, ClinoReactor® can be kept for maximum 3 days at 4°C in the inner package. Note: close the hole from the needle with tape, to prevent moisture loss and contamination.

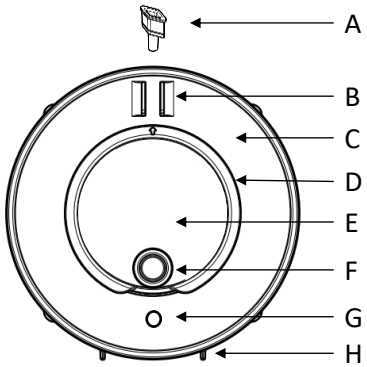

Figure 1 ClinoReactor® for single use. (A) Top plug enables media dispensing and removal. **(B) Vents** to ensure correct gas exchange and humidification in the culture chamber. **(C) Humidification chamber** containing the unhydrated humidification beads. **(D) Petri dish lid** for opening the entire culture chamber in a petri dish fashion. **(E) Cell culture chamber**. **(F) Front port** giving access to the culture chamber. **(G) Hydration port** for hydration of the humidification beads with sterile water. **(H) Feet** allowing the ClinoReactor® to stand upright.

Equilibration of the culture chamber

1. Unwrap the ClinoReactor® from the inner package and place standing up.
2. If residual water from the hydration is visible use a tissue or paper towel to remove it.
3. Remove the top plug (**Figure 1 A**) and add 5-6 mL sterile water to the cell chamber and wash by rotating the ClinoReactor® in your hand.
4. Remove the water and repeat the washing step (Step 3).
5. Remove the top plug (**Figure 1 A**), remove the water with a syringe, and fill the cell culture chamber with 5-6 mL media, leaving an aircushion. Replace the top plug and sterilize the area around the port with 100-200 µL 70 % Ethanol solution.
6. Place the ClinoReactor® with media in ClinoStar® rotating at **15 RPM for minimum 2 hours.** Alternatively, the ClinoReactor® can be placed on a shaker at low speed.

Placing ClinoReactor in the ClinoStar

ClinoReactors can be placed on any of the six axle holders in the incubation chamber. The ClinoReactor® is specifically designed to easily snap into place and stay securely fastened.

To insert a ClinoReactor® simply place it directly in front of a holder and give it a little push until you hear a "click" (**Figure 2**).

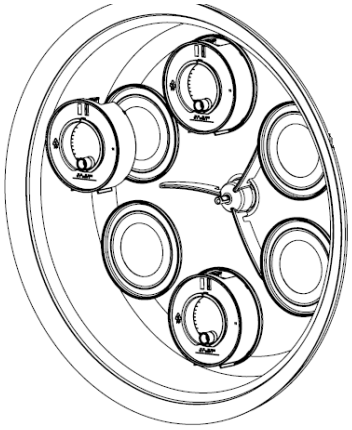

Figure 2 Insert ClinoReactor® into ClinoStar®

ClinoReactors can be attached while the axle is rotating or stopped.

This means that you do not need to touch the tablet to stop the axle before attaching or removing the ClinoReactor®.

After reinserting the ClinoReactor® use of the disperse function on the tablet will help you to get an even distribution of your 3D cell constructs within the ClinoReactor®.

You should regularly adjust the speed of the ClinoReactor®, for guidance please consult our protocol:

"Speed adjustment in ClinoReactor"

Using the provided tool

The ClinoReactor toll has been developed for making the handling of the ClinoReactors an ergonomic good experience and for reducing the risk of contamination. The lid and ports of the ClinoReactor can be opened by lifter tool (**Figure 3**) provided with the box. The tool has two functions: 1) opening lid, front port and the top port without ergonomic stress 2) securing of the lid and ports for easy handling and replacement with gloves.
To **open the petri dish lid**, place the opener on top the top of the vent and the edge of the tool under the collar of the lid and press downwards. The lid will now open. When closing the lid again, please ensure that you hear two "clicks", press on top and bottom of the lid. The **front port is opened** by sliding the horseshoe shaped part of the tool into the collar of the front port and pulling up as showed in figure 3. The front port is now securely fastened into the tool and can be placed on the table for clean handling. To secure and open the **top port** in the tool slide the V-shaped part on to the top port to fasten, the top port can be removed and placed on the table.

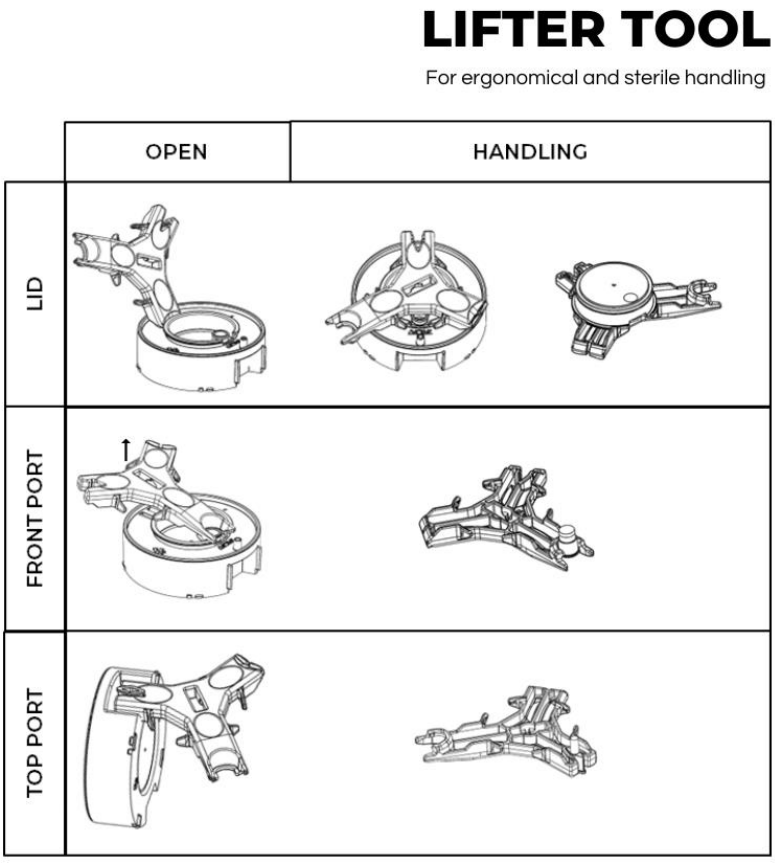

Figure 3: Lifter tool used for opening and handling lid and ports of the ClinoReactor®

Warranty/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.

For additional product or technical information visit www.celvivo.com or consult CelVivo Aps at info@celvivo.com or +45 70 228 228.


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