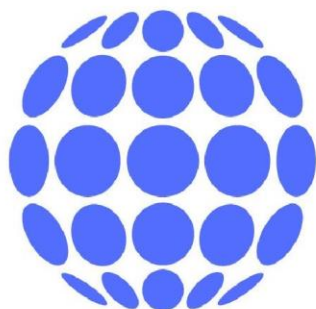




CELVIVO
STRESS-FREE 3D™

Product Information

ClinoReactor®

ClinoReactor®

Product Introduction

The rotation of the ClinoReactor® keeps cells and 3D structures suspended in a shear stress-free environment, promoting strong cell-cell interactions. Supporting the maintenance (or recovery) of tissue-like functions.

The ClinoReactor® facilitates rapid gas exchange between the growth medium and the surrounding atmosphere, ensuring an adequate oxygen supply for the cells and maintaining pH balance in bicarbonate-buffered media.

As the ClinoReactor® rotates, the movement of the vessel transfers motion to the growth medium, causing it to rotate as well. The extent to which the medium follows this motion depends on its viscosity, which influences the shear force within the system. Suspended 3D structures experience two main forces: the viscosity-driven flow of the medium around them and the pull of gravity.

Equilibrium is reached when the upward force generated by the movement of the medium counterbalances the downward pull of gravity. At this point, 3D structures remain in a near shear stress-free suspension. This minimal shear stress is crucial for facilitating cell-cell attachment. A slight flow of medium around the structures helps minimize the diffusion depletion zone.

Because gravity acts more strongly on larger structures, as they displace more volume, a higher rotational speed is required to keep them suspended. In a population with varying structure sizes, a speed sufficient for smaller structures may not prevent larger ones from sinking at the bottom. This variation in size can lead to increased shear forces on certain structures. Therefore, maintaining uniform size and shape among 3D structures is highly beneficial, ensuring they all 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.

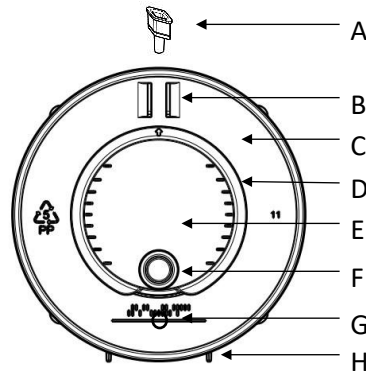


Figure 1 ClinoReactor® for single use (A) Top plug enables media dispensing and removal. **(B) Vents** to ensure correct gas exchange and humidification in the culture chamber. **(C) Humidification chamber** containing the non-hydrated humidification beads. **(D) Petri dish lid** for opening the entire 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 CO₂, temperature, and potentially O₂ levels in alignment with the conditions inside 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 while preventing media evaporation.

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 organoids or spheroids by removing the lid **(D)**. When closing the petri dish lid, it 'click's twice when closed properly. The front port **(F)** is used to add single cells or spheroids directly into the culture chamber.

The humidification beads are 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. Expiration date is printed onto the ClinoReactor® packaging.

Product Information

Product Name	Catalog #	Size	Description/Use
ClinoReactor®	10004-1	12 x ClinoReactors	ClinoReactors® with a 10 mL culture chamber.
WFI water ampoules		12 x 25 mL	Water ampoules for hydrating blue humidification beads.
Lifter tool		1 Lifter tool	Port opening and closing tools for ClinoReactors®.
Black contrast plate		1 Black contrast plate	Black Plate for better visibility when handling constructs in the ClinoReactors®.
Plugs (sterile)		4 x Spare top plugs (sterile)	Spare top plugs available in case of loss or contamination.

Directions for Use

This protocol describes how to prepare a ClinoReactor® vessel for use. 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 (Basal medium without additives is sufficient)
- 70 % Ethanol solution

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 5–6 mL of media, the air cushion helps eliminate any air bubbles that may be present in the chamber.

Protocol

Hydration of the humidification beads

1. Transfer the ClinoReactor® to the sterile workspace.
2. Open the first ClinoReactor® package.
3. Place the ClinoReactor® lying down on a flat surface with the front port facing upward.
4. Collect all the hydration beads at the bottom of the ClinoReactor®.
5. Hydrate the humidification beads by adding 25 mL sterile water through the hydration port by piercing the inner package (**Figure 1 G**).
6. Keep the ClinoReactor® with the front facing upwards. Wait minimum **4 hours** until the humidification beads have absorbed the water.
7. Close the hole from the needle with tape to prevent moisture loss and contamination before removing the ClinoReactors from the sterile workspace.

Note: After hydration of the beads, ClinoReactor® can be kept for maximum 10 days at 4°C in the inner package.

Equilibration of the culture chamber

1. Unwrap the ClinoReactor® from the inner package and place standing up.
Note: If residual water from the hydration runs out use a tissue or paper towel to remove it.
2. Remove the top plug (**Figure 1 A**)
3. Add 5-6 mL sterile water to the cell chamber.
4. Wash by rotating the ClinoReactor® in your hand.
5. Carefully remove the water using a syringe or a Pasteur pipette, ensuring the membrane is not touched.
6. Repeat steps 3 to 5.
7. Fill the cell culture chamber with 5-6 mL media, leaving an aircushion.
8. Close the ClinoReactor® by replacing the top plug.
9. Sterilize the area around the port with 100-200 µL 70 % Ethanol solution.
10. Place the ClinoReactor® with media in ClinoStar® rotating at **15 RPM for minimum 2 hours**.

Note: 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**).

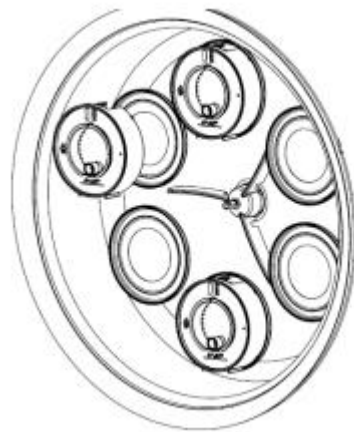


Figure 2 Insert ClinoReactor® into ClinoStar®

ClinoReactors can be attached while the axle is rotating or stopped. This means you do not need to manually stop the axle via the tablet before attaching or removing the ClinoReactor®.

After reinserting the ClinoReactor®, use the speed adjustment function to achieve an even distribution of your 3D cell constructs. Regular speed adjustments are recommended. For guidance, please refer to our protocol: "Speed Adjustment in ClinoReactor®" and Figure 3.

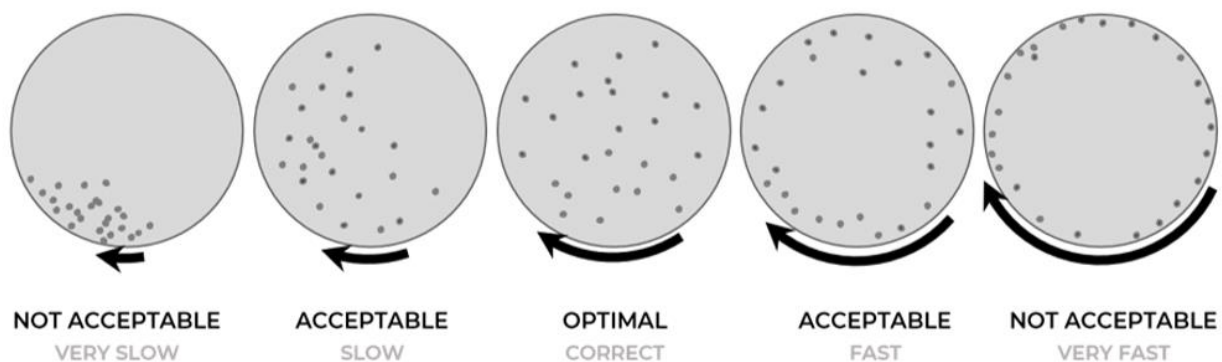


Figure 3: Speed adjustment

Using the provided tool

The ClinoReactor® tool has been designed to improve the ease of handling ClinoReactors® while minimizing the risk of contamination. The provided lifter tool (**Figure 4**) allows for the easy opening of the lid and ports, ensuring both convenience and sterility.

This tool serves two main functions:

- Opening the lid, front port, and top port with minimal strain.
- Securing the lid and ports for easy handling.

Using the ClinoReactor® Tool

- *Opening the Petri Dish Lid:* Place the opener on top of the vent, position the edge of the tool under the collar of the lid, and press downward. The lid will open. When closing the lid, ensure you hear two distinct “clicks” by pressing both the top and bottom of the lid.
- *Opening the Front Port:* Slide the horseshoe-shaped part of the tool under the collar of the front port and pull upward, as shown in **Figure 4**.
- *Opening the Top Port:* To secure and remove the top port, slide the V-shaped section of the tool onto the port until it locks into place.

LIFTER TOOL

For ergonomical and sterile handling

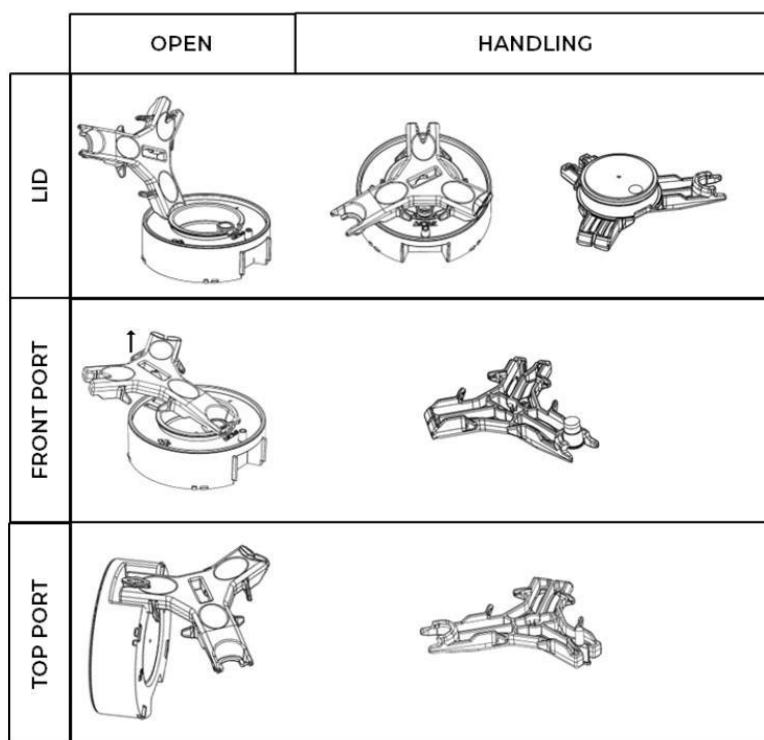
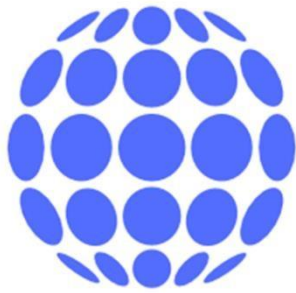


Figure 4: Lifter tool used for opening and handling lid and ports of the ClinoReactor®

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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