

# Preparation of ClinoReactor

## Protocol

The ClinoStar® system is an *in vitro* system to grow primary and immortal cell lines to retain and recover their *in vivo* physiological attributes. The spheroids or organoids are grown in the ClinoReactor® which is an advanced disposable bioreactor. This protocol describes how to prepare a ClinoReactor® vessel for use in experiments. ClinoReactor® is delivered in a double wrapped packaging 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 5-6 mL of media, the aircushion will assist in removing air bubbles that could be in the chamber.

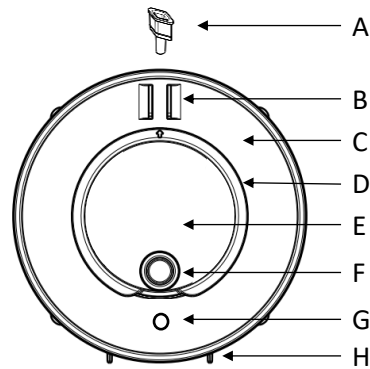
### Protocol

#### 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.

#### 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.



**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.

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](http://www.celvivo.com) or consult CelVivo Aps at [info@celvivo.com](mailto:info@celvivo.com) or +45 70 228 228.

