

Gardening Spheroids and Organoids

Protocol

During formation of spheroids or organoids the constructs can form irregular shapes or sizes. To ensure uniformity, optimal growth and reduce the risk of clumping it is necessary to remove/reduce the irregularities. This procedure is referred to as “gardening” and it is recommended to do simultaneously with a media change or when transferring spheroids or organoids to a new ClinoReactor®. This procedure is performed more often for the young spheroid cultures. It will ensure that uniform spheroid populations enter the experimental set up. It should be noted that the spheroid shape and size differences could be attributed to the experimental setup and such should be noted and handled accordingly to experimental set up.

Reagents and Materials

- Cell culture media with supplements
- 70 % Ethanol solution
- 10 mL syringe with a needle (e.g. 18Gx2”)
- 50 mL sterile tubes
- 200 µL wide bore/cut pipette tips
- 1000 µL wide bore/cut pipette tips

Additional information

To avoid unnecessary disturbance of the spheroids it is recommended to remove irregular or sizable spheroids or organoids, following a media change or transfer to a new bioreactor. These procedures are described in detail in here: [007_Protocol_Transferring_Spheroids_or_Organoids](#) or [004_Protocol_Cell_Culture_Media_Change_ClinoReactor](#).

During the procedure it is immensely important to avoid infections, therefore do not touch any of the material e.g., plugs or caps, that are in direct contact with the cell culture and be sure to work as sterile as possible.

Protocol

1. Prepare the workspace with tubes, waste container, pre-heated cell culture media and syringes in a sterile environment.
2. Collect the ClinoReactor® from the ClinoStar®, place it upright in the sterile environment, and wait for the spheroids to settle.
3. Open the top plug (**Figure 1 A**) and aspirate 2-3 mL of the cell culture media and replace the plug.
4. Place the ClinoReactor® flat on a sterile surface.
5. Remove the lid to the cell culture chamber (**Figure 1 D**), place the lid on a sterile surface.
6. Carefully with a wide bore/ cut tip aspirate and discard the irregular constructs. (The irregular constructs can be collected into petri dish for further examination, use 200 µL or 1000 µL tips depending on cluster size)
7. Replace the lid to the cell culture chamber and place the ClinoReactor® in an upright position.

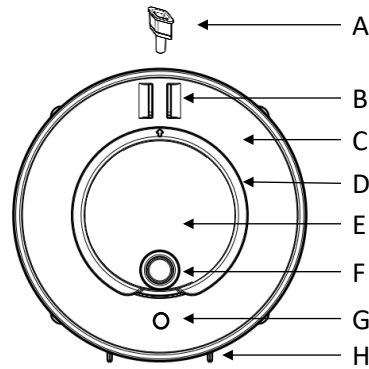


Figure 1 ClinoReactor® for single use (A) Top plug enables media dispensing and removal. **(B) Vents** to ensure correct gas exchange and humidification in the culture chamber. **(C)** Humidification chamber containing the unhydrated humidification beads. **(D) Petri dish lid** for opening the entire 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.

8. Wait for the spheroids or organoids to settle at the bottom and open the top plug (**Figure 1 A**).
9. Slowly refill the ClinoReactor® with fresh preheated media without disturbing the spheroids. Cell culture media should be clearly visible in the collar.
10. Remove air bubbles trapped in the cell culture chamber by gently tapping the ClinoReactor® (Please refer to **004_Protocol_Cell_Culture_Media_change_ClinoReactor** for detail description of media refill and possible bubble removal).
11. Close the chamber by placing the plug (**Figure 1 A**) into the valve.
12. Remove any remaining cell culture media from the collar around the plug (**Figure 1 A**)
13. Disinfect the collar with 100 μ L 70% Ethanol solution.
14. Aspirate the 70 % Ethanol solution and place the ClinoReactor® in ClinoStar®.

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.

