Cell Culture Media Change in a ClinoReactor

Protocol

The ClinoReactor® is a bioreactor, when combined with the ClinoStar® provide an easy-to-use system for generation of 3D cell structures referred to as spheroids or organoids. Spheroids or organoids are, compared to conventional 2D cell culture, widely recognized as a more physiologically relevant system. Spheroid or organoid formation depend on several factors such as cell line and growth rate, therefore, assay specific optimization of the conditions is recommended. This protocol describes how to change cell culture media in the ClinoReactor® for both organoids and spheroids, but for convenience the word “spheroid” is used.

Reagents and Materials

- Cell culture media with supplements
- 70 % Ethanol solution
- 10 mL syringe with a needle (e.g. 18Gx2”)
- 50 mL sterile tubes

Additional information

For information on preparation and equilibration of the ClinoReactor® please refer to **003_Protocol_Preparation_Of_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.

Protocol

1. Prepare the workstation with tubes, waste container, pre-heated cell culture media and syringes in a sterile environment.
2. Aspirate 11 mL of fresh warm cell culture media in the syringe and place it in a 50 mL centrifuge tube.

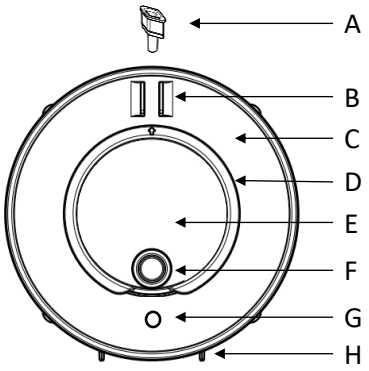
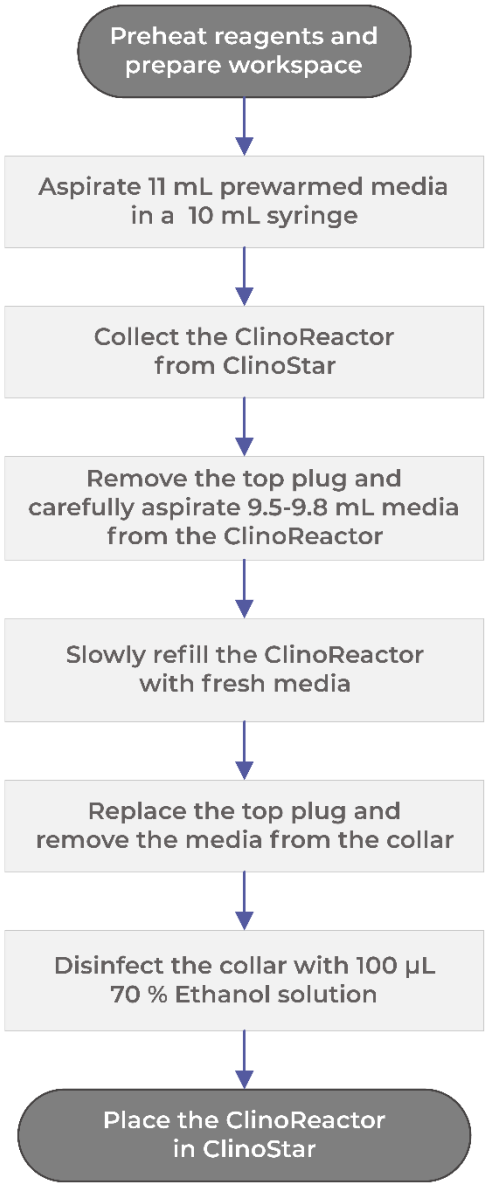


Figure 1 ClinoReactor® for single use (A) Top plug enables media dispensing and removal. **(B) Vents** to ensure correct gas exchange and humidification in the culture chamber. **(C)** Humidification chamber containing the unhydrated humidification beads. **(D) Petri dish lid** for opening the entire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

3. Collect the ClinoReactor® from the ClinoStar® and place it in the sterile environment NOTE: It is essential that the workspace including all necessary accessories is prepared prior to moving the ClinoReactor® from ClinoStar® into the working space.
4. Place the ClinoReactor® in vertical position and wait until spheroids settle on the bottom of the vessel (usually 10 to 40 seconds depending on size and compactness of the spheroids).
5. Remove the top plug to the cell culture chamber (**Figure 1 A**), place it on a sterile surface.
6. Carefully remove the cell culture medium (approximately 9.5—9.8 mL) with a syringe without disturbing the spheroids. (Placing the tip of the needle/tips just under the liquid surface and moving it down while aspirating will prevent unintentional spheroid removal).
7. Slowly refill the ClinoReactor® with media without disturbing the spheroids. Cell culture media should be clearly visible in the collar. NOTE: placing the needle directly against the front or back of the ClinoReactor® wall disperse the medium and create more gentle re-filling.
8. Remove air bubbles trapped in the cell culture chamber by gently tapping the ClinoReactor® against the table.
9. Close the chamber by placing the top plug (**Figure 1 A**) into the valve.
10. Remove any remaining cell culture media from the collar around the plug (**Figure 1 A**).
11. Check for any remaining bubbles by careful rotating the vessel. If no bubbles could be observed proceed directly to step 14.
12. If the air bubbles are present in the cell culture chamber place the ClinoReactor® in vertical position, open the top plug (**Figure 1 A**), then add a small amount of fresh medium to overfill the cell culture chamber to the point where medium fills the collar around the port. Remove air bubbles trapped in the cell culture chamber by gently tapping the ClinoReactor®.
13. Close the chamber by placing the top plug (**Figure 1 A**) into the valve.
14. Disinfect the collar with approximately 100 µL 70 % Ethanol solution.
15. 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.