Transferring Spheroids and Organoids

Protocol

In the ClinoStar® system spheroids created from immortal cell lines often regain their original functionality after approximately 18 days. During this time cell culture media needs to be exchanged every time its nutrient and buffering capacity reach critical levels. The frequency of media changes will depend on cell and medium type. For C3A cell line (human hepatoma cell line) grown in physiological glucose level media the medium change schedule would look as follows: 48h / 48h / 72h / 48h / 48h / 72. The ClinoReactor® is single use and should be changed every 10 days. If the ClinoReactor® is used for a longer period, the membrane on the circumferential side will be in risk of clotting. This membrane allows gas exchange and ensure the media oxygen and buffering system is kept at a constant volume, if clotted or blocked the spheroids or organoids inside the ClinoReactor® will deteriorate.
These procedures should be optimized according to the individual cell line and downstream applications.

Reagents and Materials

- Cell culture media with supplements
- Equilibrated ClinoReactor®
- 70 % Ethanol solution
- 10 mL syringes 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

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.

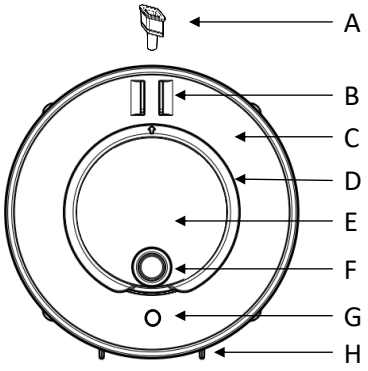
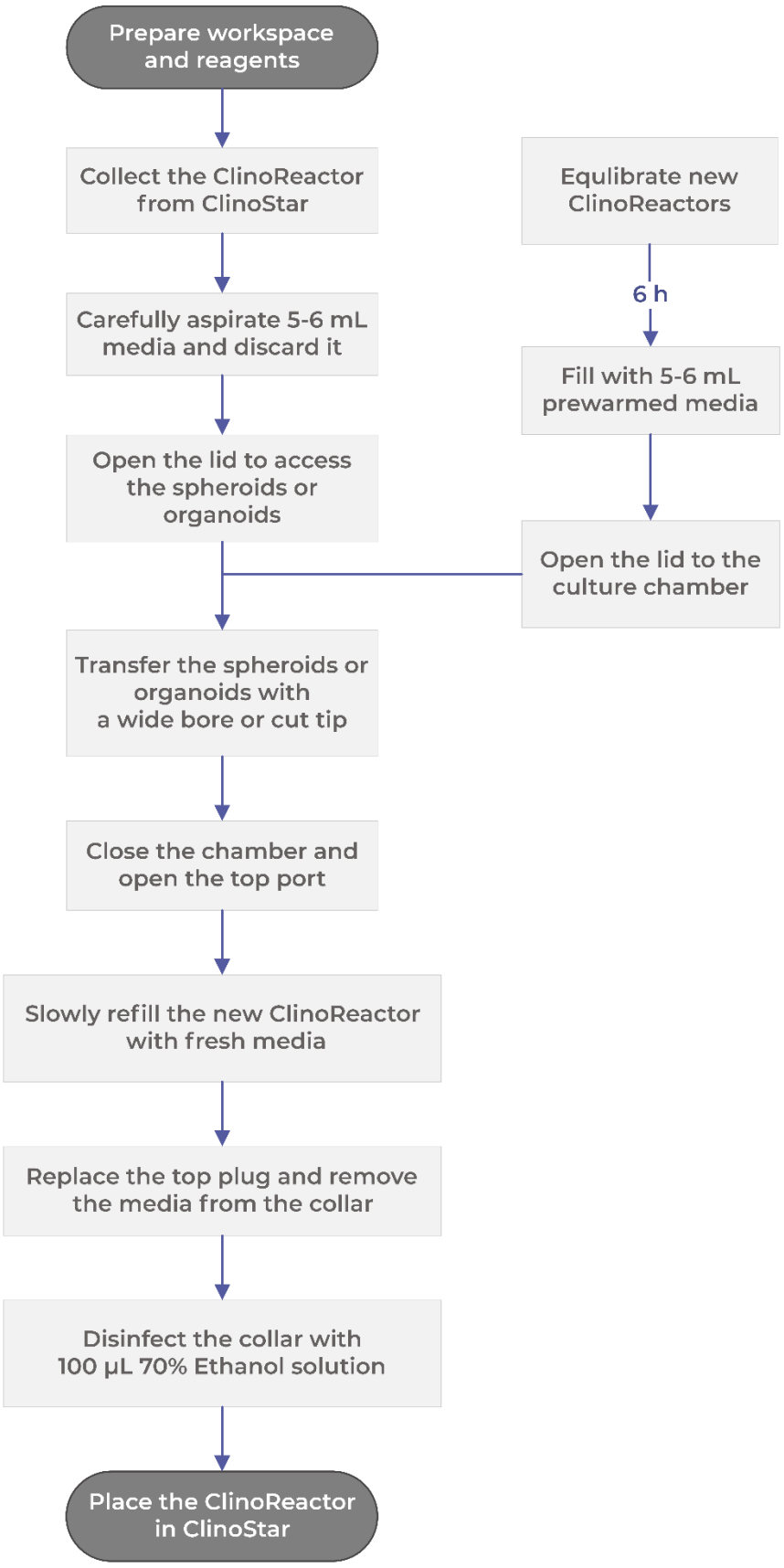


Figure 1 ClinoReactor® for single use (A) Top plug enables media dispensing and removal. **(B) Vents** to ensure correct gas exchange and humidification in the culture chamber. **(C)** Humidification chamber containing the unhydrated humidification beads. **(D) Petri dish lid** for opening the entire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

Protocol

1. After 10 days the ClinoReactor® should be exchanged and the spheroids or organoids transferred to a new vessel. This is done to avoid clotting of the membrane on the circumferential side, essentially blocking CO_2 exchange and killing the cell culture.
2. The day prior to the transfer, equilibrate a suitable number of ClinoReactors according to protocol: **003_Protocol_Preparation_of_ClinoReactor**.
3. Collect the equilibrated ClinoReactors from the ClinoStar® and transfer them to the sterile workspace.
4. Prepare the new ClinoReactors with 5-6 mL warm media. Place the ClinoReactor® flat on a sterile surface.
5. Collect the ClinoReactor® containing the spheroids or organoids and transfer it to the sterile workspace, place it upright and wait for the spheroids to settle at the bottom.
6. Remove the top plug to the cell culture chamber (**Figure 1 A**) and place the plug on a sterile surface.
7. Aspirate 5-6 mL of the cell culture media and discard it.
8. Replace the plug and place the ClinoReactor® flat on a sterile surface.
9. Spheroids or organoids can be removed and loaded to the new ClinoReactor® via the sampling port (**Figure 1 F**) or by opening the entire cell culture chamber (**Figure 1 D**).
10. Use a wide bore/cut tip to carefully move the spheroids or organoids to the new ClinoReactor® prefilled with 5-6 mL warm media.
11. Close the port or lid to the ClinoReactor® and place it upright.
12. Remove the top plug (**Figure 1 A**).
13. Slowly fill the culture chamber in the ClinoReactors with fresh preheated media. Overfill the chamber making media visible in the top collar.
14. Replace the top plug into the valve and close the chamber (**Figure 1 A**) (please refer to **004_Protocol_Cell_Culture_Media_Change_ClinoReactor** for detail description of media refill and possible bubble removal).
15. Remove any remaining cell culture media from the collar around the top plug (**Figure 1 A**).
16. Disinfect the collar with 100 µL 70 % Ethanol solution.
17. Aspirate the 70 % Ethanol solution and place the ClinoReactor® in ClinoStar® at 37°C and 5 % CO_2, the rotation speed is correct when the spheroids or organoids are in a stationary orbit.

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


Prepare workspace and reagents
Collect the ClinoReactor from ClinoStar
Carefully aspirate 5-6 mL media and discard it
Open the lid to access the spheroids or organoids
Transfer the spheroids or organoids with a wide bore or cut tip
Close the chamber and open the top port
Slowly refill the new ClinoReactor with fresh media
Replace the top plug and remove the media from the collar
Disinfect the collar with 100 µL 70% Ethanol solution
Place the ClinoReactor in ClinoStar
Equlibrate new ClinoReactors
6 h
Fill with 5-6 mL prewarmed media
Open the lid to the culture chamber