Preperation of Spheroids and Organoids from Single Cell Suspension

Protocol

As an alternative to force aggregation methods e.g. cell aggregation with low adherent microwell plates, the spheroids or organoid can be prepared from a single cell suspension. This protocol describes how to create spheroids or organoids from a single cell suspension via self-assembly. This protocol has been used for human HEPG2/C3A cells and rat INS-1E cells. Depending on the cell line, adhesion to the culture plates, and growth rate we recommend optimisation of the conditions to accommodate your needs.

Reagents and Materials

- Equilibrated ClinoReactor®
- Cell culture media
- Cell counter or hemacytometer
- Single cell suspension

Additional information

The general principle for cultivating spheroids is described in **001_Protocol_Cultivating_Spheroids_and_Organoids** with references to relevant protocols. The cells used for the procedure are often 70-90 % confluent, see protocol **002_Protocol_Sub_Cultivating_Cells_in_2D** which describes sub-cultivation of cells. The day prior to the experiment initiate the equilibration of the ClinoReactor® see protocol **003_Protocol_Preparation_of_ClinoReactor**. For HEPG2/C3A and INS-1E cells it is recommended to do the first medium change 48 hours after the self-aggregation procedure was started. Medium change procedure is described in **004_Protocol_Cell_Culture_Media_Change_ClinoReactor**. NOTE: Be careful not to puncture the filter of the ClinoReactor with the needle.

Protocol

1. Prepare a single cell suspension of the desired cell type.
2. Remove the media from the equilibrated ClinoReactor® via the top port (**Figure 1 A**).

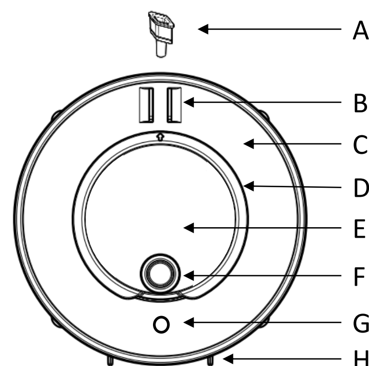
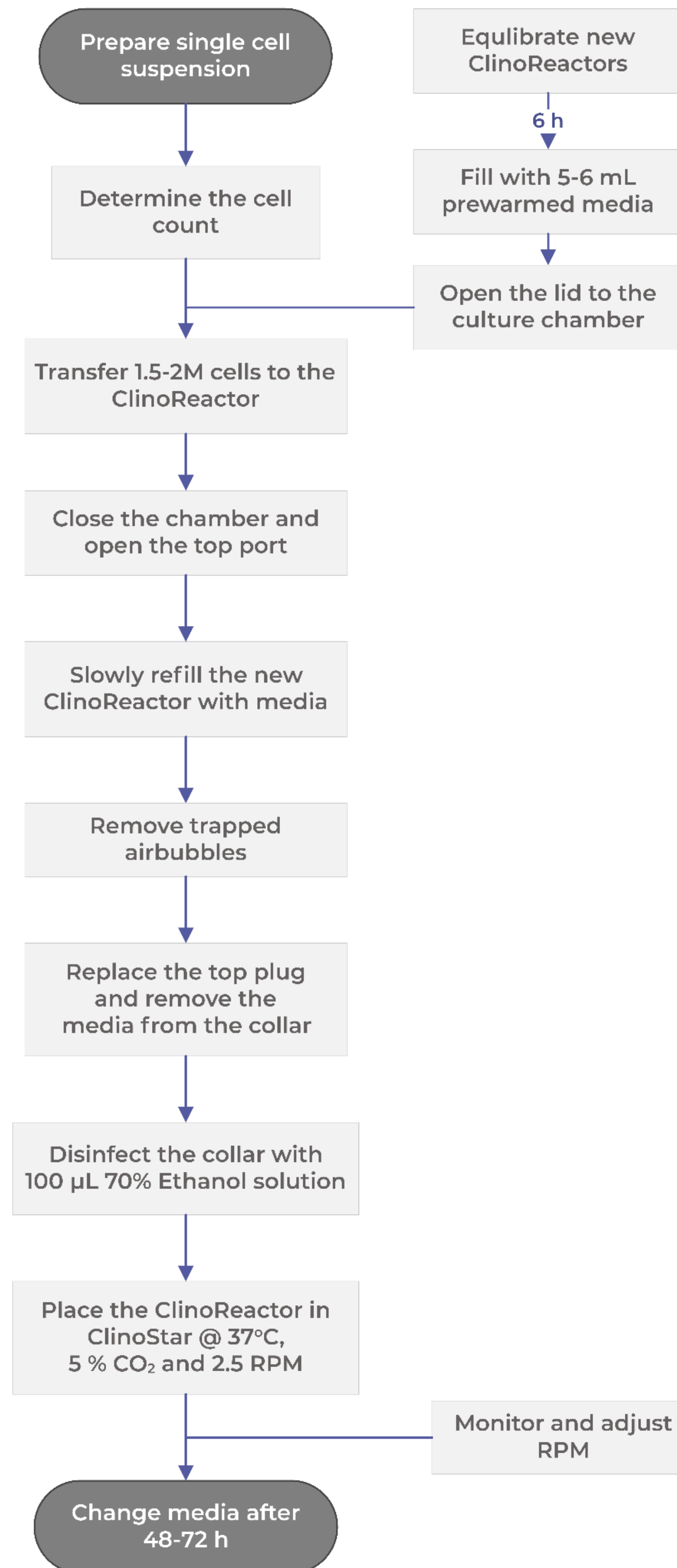


Figure 1 ClinoReactor® for single use (A) Top plug enables media dispensing and removal. **(B) Vents** to ensure correct gas exchange and humidification in the culture chamber. **(C)** Humidification chamber containing the unhydrated humidification beads. **(D) Petri dish lid** for opening the entire 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. Fill the chamber with 5-6 mL prewarmed cell culture media.
4. Close the ClinoReactor® and gently place it flat on the surface.
5. Determine the cell count in the single cell suspension.
6. Transfer an amount corresponding to 1.5 -2 million cells to the equilibrated ClinoReactor® through the sample port (**Figure 1 F**) or via the cell culture chamber (**Figure 1 D**).
7. Close the bottom port (**Figure 1 F**) and place the ClinoReactor® upright.
8. Remove the top plug (**Figure 1 A**).
9. Slowly refill the ClinoReactor® with media without disturbing the cells. Cell culture media should be clearly visible in the top collar. NOTE: placing the needle end directly against the front or back ClinoReactor® wall disperse the medium flow and create more gentle re-filling.
10. Overfill the chamber with media. The media should be visible in the top collar.
11. Remove air bubbles trapped in the cell culture chamber by gently tapping the ClinoReactor®.
12. Replace the top plug (**Figure 1 A**) and remove the excess media with a pipette.
13. Check for any remaining bubbles by careful rotating the vessel. If no bubbles could be observed proceed directly to step 15.
14. If the air bubbles are present in the cell culture chamber place the ClinoReactor® in a 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®.
15. Sterilize the area around the plug with 70 % Ethanol solution.
16. Incubate the ClinoReactor® in ClinoStar® at 37°C, 5 % CO_2 and with an initial rotation of 2.5 RPM. The speed should be optimised according to individual cell lines. An initial speed to fast or slow could result in cell clumping.
17. Observe the ClinoReactor® during the first 48 hours for sizable cell clump formation. If present, remove the clump from the ClinoReactor® (see **006_Protocol_Gardening_Spheroids_and_Organoids**).
18. It is recommended to change the media for the first time after 48-72 hours.
19. The rotation speed should be monitored daily and adjusted to keep the spheroids or organoids in a stationary orbit. When using the ClinoStar® camera cell conglomerates of as little as 300 cells should be visible inside the ClinoReactor® vessel.
20. With time, the shape of organoids or spheroids will become more spherical and after approximately 3 weeks the spheroid or organoid population should contain similar-sized, round spheroids. If the size varies too much it is possible to select spheroids or organoids of similar size.

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.


Prepare single cell suspension
Equlibrate new ClinoReactors
6 h
Fill with 5-6 mL prewarmed media
Determine the cell count
Open the lid to the culture chamber
Transfer 1.5-2M cells to the ClinoReactor
Close the chamber and open the top port
Slowly refill the new ClinoReactor with media
Remove trapped airbubbles
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 @ 37°C, 5 % CO_2 and 2.5 RPM
Monitor and adjust RPM
Change media after 48-72 h