CelCradle-500 Technical Report XVIII

Cultivation of 3T3 cells

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1 Description

CelCradle-500 provides a powerful tool to achieve high cell density and high productivity of target bioproducts in a cell culture because it has a unique feature of offering high oxygen transfer and low shear stress culture environment. Users can easily collect highly concentrated cells, virus or non-secreted products from one CelCradle-500 bottle. In this study, the application of CelCradle-500 for growth of 3T3 cells is illustrated. The following experiments were performed by culturing 3T3 cells (ATCC CCL-92) in CelCradle-500 for 168 hours. Final 7.3 × 108 cells were achieved in one CelCradle-500 bottle. The cells don't form 3-D tissue structure as other cell lines and that's the major reason to achieve lower cell density than the other cell lines. This technical sheet provides a general protocol for users to start up their culture. However, the optimum condition of each cell culture for each case may require the users to determine.

2 Material

Device	Cell Line	Medium	Seed
CelCradle-500	3T3, ATCC CCL-92	DMEM/10%FBS	1.0 x 10^8 cells/bottle

3 Protocol

3.1 Inoculum Preparation

Prepare one roller bottle with sub-confluence cells. Harvest cells by standard trypsinization protocol. Prepare 5.0×10^7 to 1.0×10^8 suspend cells and concentrate cells in 30 ml culture medium.

3.2 Inoculation

Pre-warm DMEM/10%FBS medium in 37℃ water bath. Take out one CelCradle-500 bottle aseptically and place in a biosafety cabinet. Open the cap and add 470 ml culture medium in each bottle. Dispense 30 ml prepared inoculum on top of the matrixes and bring to CelCradle Stage immediately. Fix the bottles on CelCradle Stage controller in CO2 incubator with 37℃, and 5% CO2 and start the compression immediately.

Immobilization

Set up immobilization parameters on the CelCradle Stage controller and start the controller by pressing "START" button. The inoculation parameters are set as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
2.0 mm/s	20 sec	2.0 mm/s	0 sec

3.3 Culture

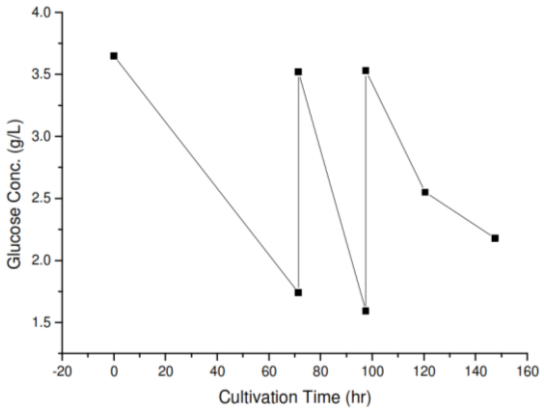
After 3 hours, reset the parameters to culture parameters as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
1.0 mm/s	2 mins	1.0 mm/s	30 sec

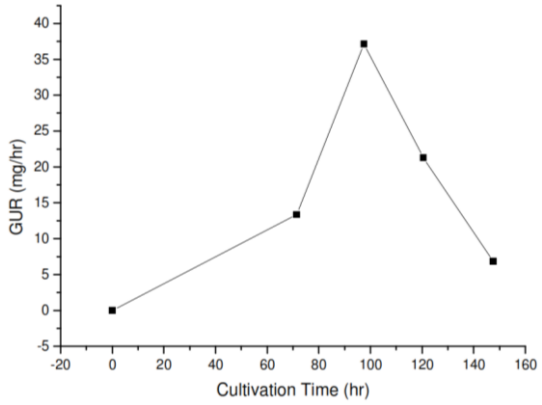
The setup parameters are only for reference. It does not necessary to be optimum parameters. VacciXcell will update recent data on the website, please check the website for updated information.

4 Result

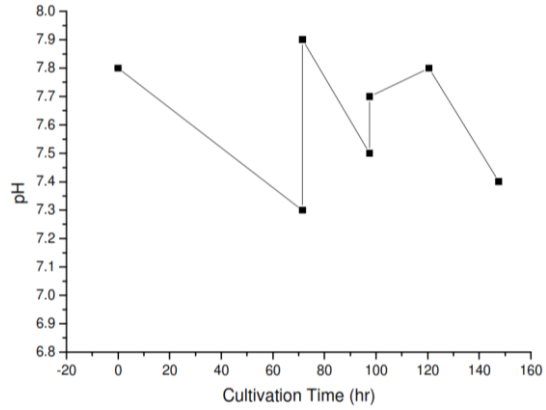
Glucose Concentration profile



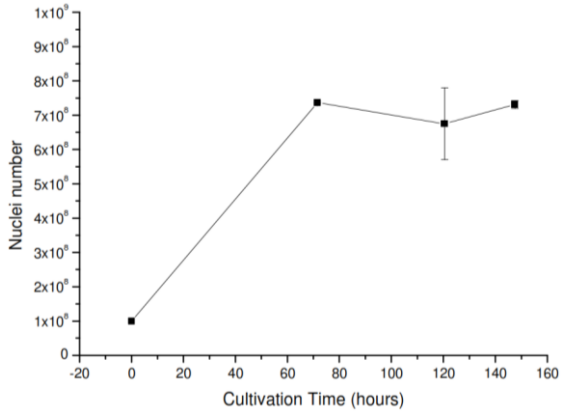
Glucose Uptake Rate (GUR, mg/hr)



pH Profile



Cell Growth Profile (by crystal violet dye nuclei count method)



The result shows that 3T3 is a kind of strong contact-inhibition cell line that is difficult to form 3D tissue structure in CelCradle. Therefore, the overall cell density in CelCradle will be limited. When cell density reaches 7.31×10^8 cells, extra cells start to dislodge from the matrix and overall cell density will keep the same. Therefore, it is suggest to seed $5X10^7$ cells and the culture should be able to reach plateau at around 3~4 days culture.

5 Summary

Seed	**Inoculum Volume**	**Medium Volume**	**Medium**
1.0 x 10^8 cells/bottle	30 ml	500 ml	DMEM/10%FBS
Total Culture Age	**Total Medium Consumed**	**Total Medium Replenish Frequency**	**Max. Cell Density**
6 days	1.5L	2 times	7.31 x 10^8 cells/bottle

6 VacciXcell Technical Support

For queries and comments, please contact the VacciXcell Technical Support team.

Email: mail@vaccixcell.com

Address: 21 Changi South Street 1, Singapore 486 777

Telephone: +65 6542 0833

Website: http://vaccixcell.com/