CelCradle-500 Technical Report V

Cultivation of VERO 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 secreted products from one 500 ml CelCradle-500 bottle. In this study, the application of CelCradle-500 for growth of Vero cells is illustrated. The following experiments were performed by culturing Vero cells in CelCradle-500 for 255 hours, and then harvested the cells from the CelCradle-500 and then sub-cultured into another CelCradle-500 bottle for 192 hours. The test is to understand the quality of the cells cultivated in CelCradle-500 system. 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	Seed for Subculture
CelCradle-500	Vero ATCC CCL-81	EX-CELL 420 (JRH)	1.04 x 10^8 cells/bottle	1.11x10^8 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 (prefer 1.0×10^8 or above) and concentrate cells in 30 ml culture medium.

3.2 Inoculation

Pre-warm M199/5%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 inoculums on top of the matrix box and bring to CelCradle immediately. Fix the bottles on CelCradle controller in CO_2 incubator with 37℃, and 5% CO_2 and start the run immediately.

Immobilization

Set up immobilization parameters on the CelCradle control box 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 4 hours, reset the parameters to culture parameters as below:

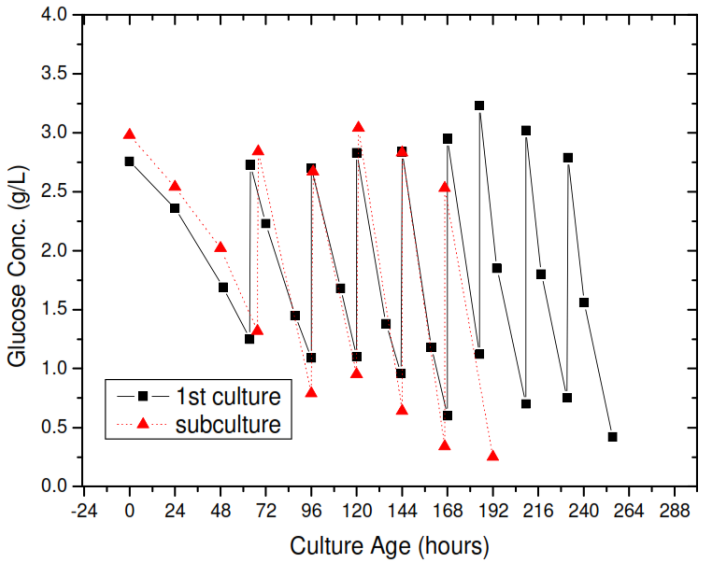
Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
1.0 mm/s	10 sec	1.0 mm/s	10 sec

Monitor the residual glucose concentration and the color of medium in order to predict the time to change culture medium. The medium was replenished started from the 3 day and then replenished one time a day until the end of the culture. Sequentially adjust the CO_2 concentration in the incubator as the pH of medium goes down. Around 10 days culture will allow the cell growth to reach plateau.

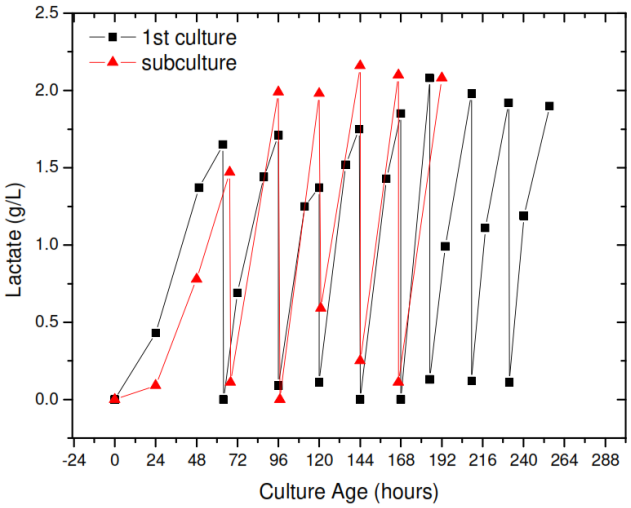
The setup parameters are only for reference. It does not necessary to be optimum parameters.

4 Result

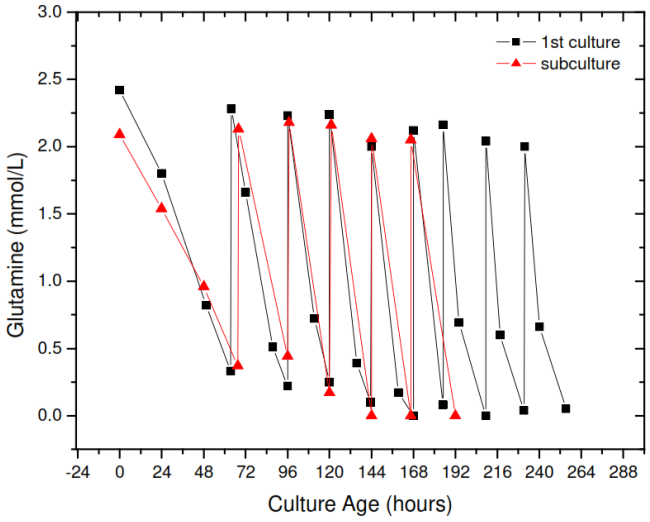
Glucose Concentration profile



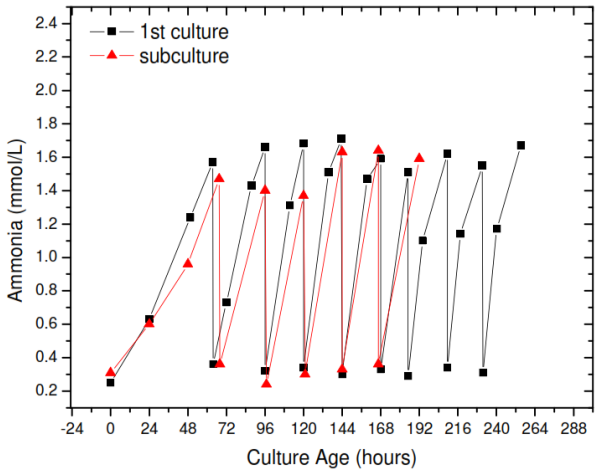
Lactate Concentration profile



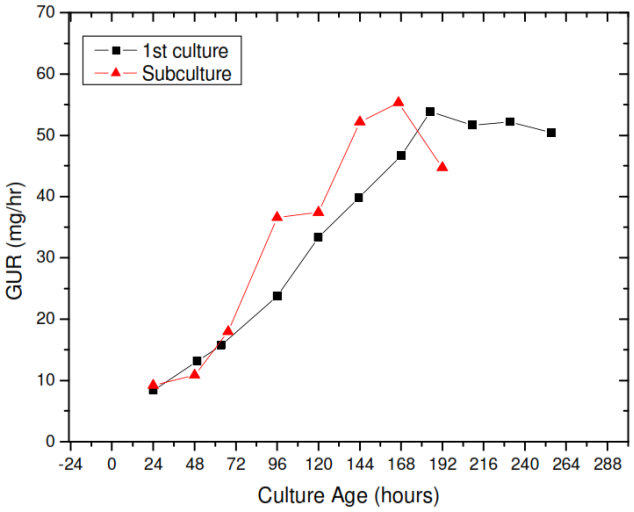
Glutamine Concentration Profile



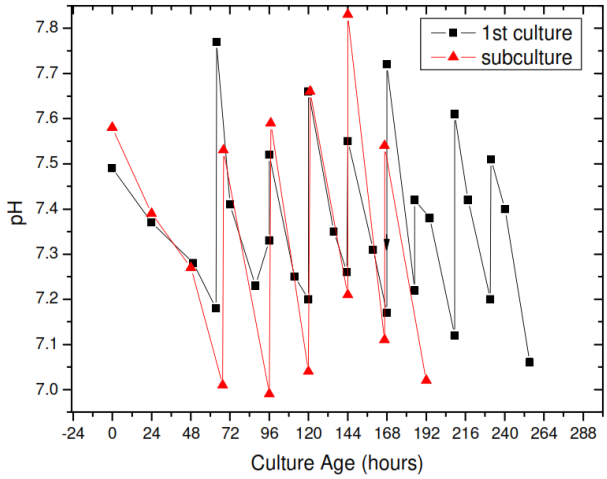
Ammonia Concentration Profile



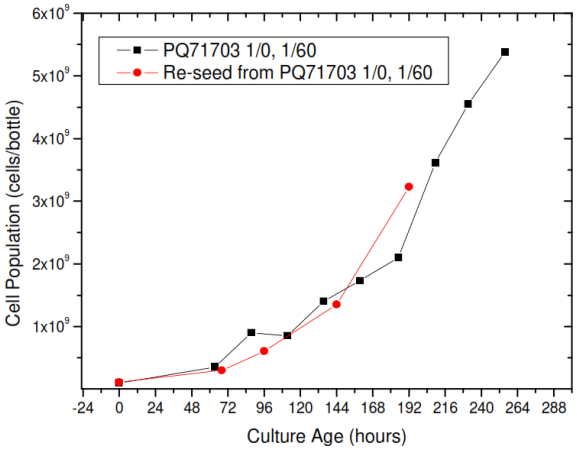
Glucose Uptake Rate



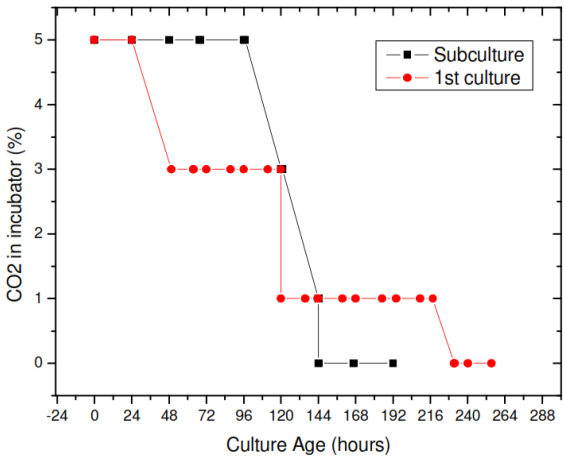
pH



Cell Growth (by crystal violet dye nuclei count method)



% of CO_2 in Incubator



The result indicates that CelCradle cell culture system can be applied in Vero cell culture. The cells harvest from the first CelCradle and subculture into the second CelCradle results a similar growth profile and metabolite profile. It indicates that the cells cultivating in CelCradle remains its activity and originality.

5 Summary

Seed	Inoculum Volume	Medium Volume	Medium
1.04 x 10^8 cells/bottle	50 ml	500 ml	M199/5% FCS
Total Culture Age	**Total Medium Consumed**	**Total Medium Replenish Frequency**	**Final Cell Density (Nuclei count)**
255 hours	4500 ml/bottle	8 times	5.4x10^9 cells/bottle

6 VacciXcell Technical Support

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