• BelloCell-500 Technical Report V •

Cultivation of VERO cells

Description BelloCell-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 BelloCell-500 bottle. In this study, the application of BelloCell-500 for growth of Vero cells is illustrated. The following experiments were performed by culturing Vero cells in BelloCell-500 for 255 hours, and then harvested the cells from the BelloCell-500 and then sub-cultured into another BelloCell-500 bottle for 192 hours. The test is to understand the quality of the cells cultivated in BelloCell-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.

Material

Device	Cell Line	Medium	Seed
BelloCell-500	Vero ATCC CCL-81	M199/5%FBS	1.04×10^8 cells/bottle
			Seed for Subculture
			1.11×10^8 cells/bottle

Protocol **Detail protocol is in General Instruction Manual*

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. **Inoculation** Pre-warm M199/5%FBS medium in 37℃ water bath. Take out one BelloCell-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 BelloStage immediately. Fix the bottles on BelloStage 3000 controller in CO_2 incubator with 37℃, and 5% CO_2 and start the run immediately.

Immobilization Set up immobilization parameters on the BelloStage 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

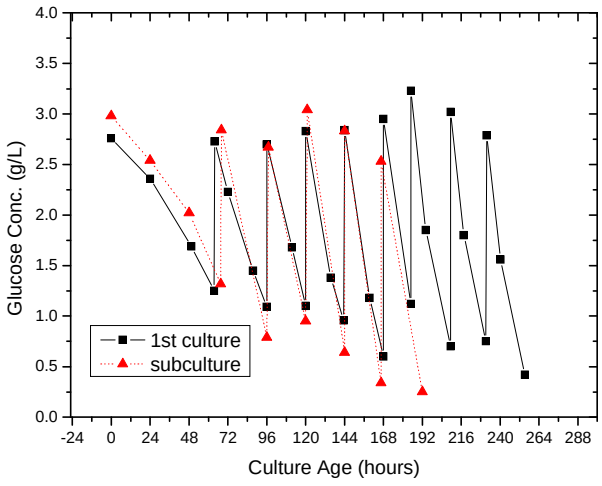
Culture After 4 hours, reset the parameters to culture parameters as below:

Up rate	Top holding time	Down rate	Bottom holding time
1.0 mm/s	10 sec	1.0 mm/s	10 sec

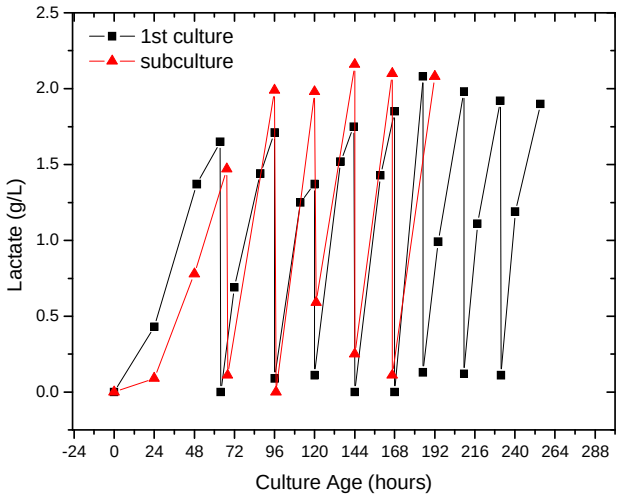
Monitor the residual glucose concentration and the color of medium in order to predict the time to change culture medium. The medium was replenished started from the 3rd day and then replenished one time a day until the end of the culture. Sequentially adjust the CO_2 concentration in the incubator as 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.*

Results

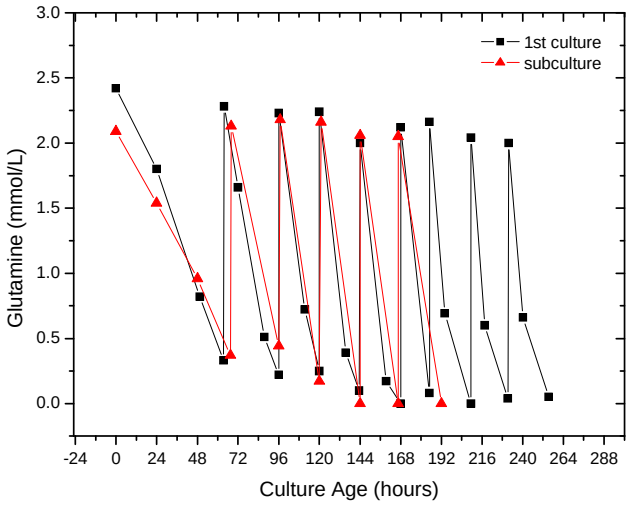
Glucose Concentration profile



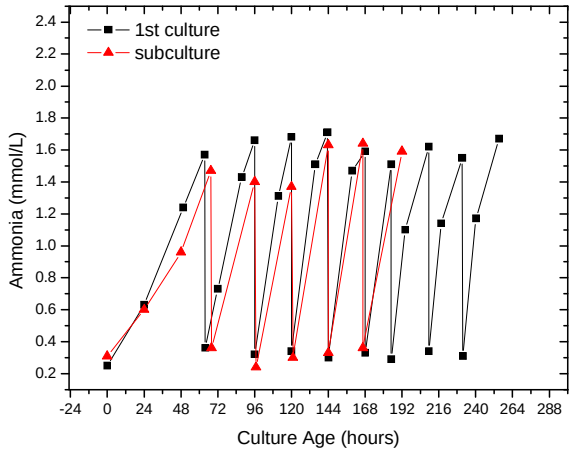
Lactate Concentration profile



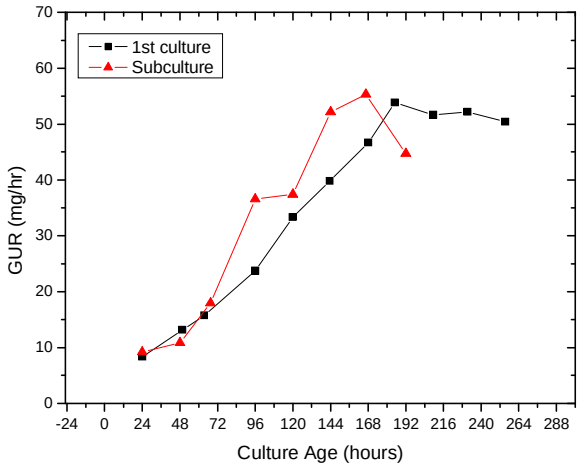
Glutamine Concentration profile



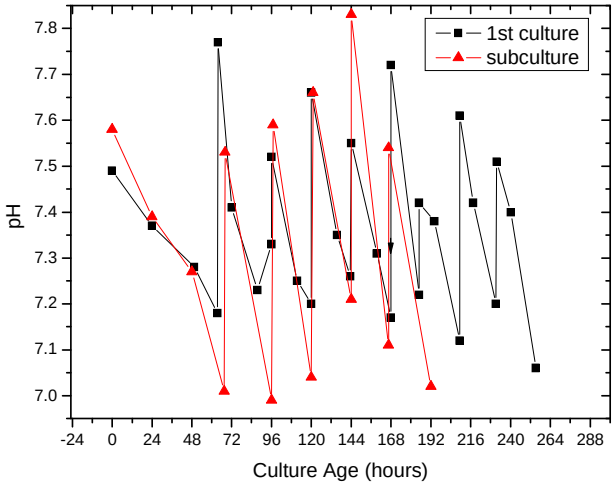
Ammonia Concentration profile



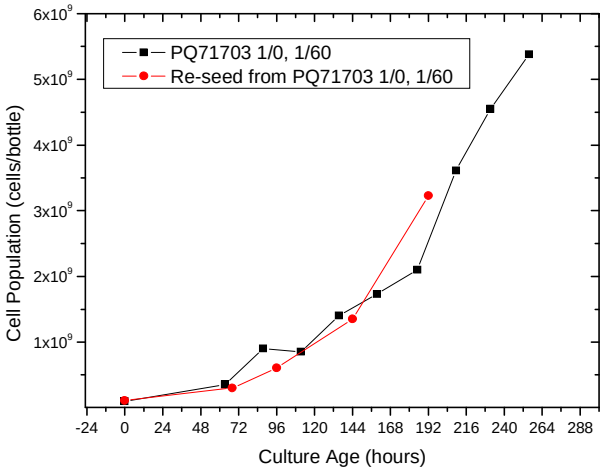
Glucose uptake rate



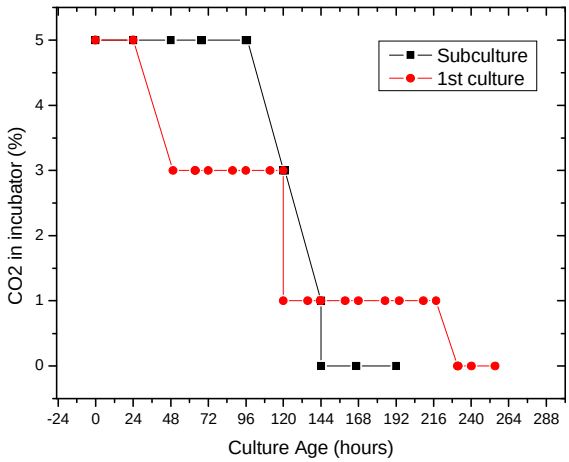
pH



Cell Growth (by crystal violet dye nuclei count method)



% of CO2 in incubator



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

Summary

Seed	**Inoculum volume**	**Medium volume**	**Medium**
1.04×10^{8} cells/bottle	50 ml/bottle	500 ml/bottle	M199/5%FCS
Total culture age	**Total medium consumed**	**Total medium replenish frequency**	**Final cell density (nuclei count)**
255 hrs	4500 ml/bottle	8	5.4×10^{9} cells/bottle

Please contact Cesco Bioengineering Technical support for any questions or comments.

http:// www.cescobio.com.tw
e-mail: info@cescobio.com.tw