- BelloCell-500 Technical Report VIII

Cultivation of HEK 293 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 HEK-293 cells is illustrated. 1.27×10^8 cells/bottle was seeded and obtain a total of 3.26×10^9 cells counted by crystal violet dye nuclei count method at 358 hours, with a total 26 folds increase of cell population. It took 7 days to grow from 1.27×10^8 cells to 2×10^9 cells. However, it took another 7 days to grow from 2×10^9 cells to 3×10^9 cells. Glucose concentration in the culture medium was monitored and kept above 1.0 g/L. 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	HEK-293	αMEM/10%FBS + 2.5 g/L glucose + 2.5 mM glutamine + 2.2 g $NaHCO_3$	1.27×10^8 cells/bottle

Protocol **Detail protocol is in General Instruction Manual*

Inoculum preparation Prepare one roller bottle. Seed with 2.5×10^7 cells total. Culture at 37℃, 5% CO_2 for total 5 days. Replenish medium at day 3rd. Harvest cells by standard trypsinization protocol. Prepare 1.27×10^8 suspend cells with viability of 97.42%, and concentrate cells in 50 ml culture medium. **Inoculation** Pre-warm αMEM/10%FBS medium in 37℃ water bath. Take out one BelloCell-500 bottle aseptically and place in a biosafety cabinet. Open the cap and add 450 ml culture medium in each bottle. Dispense 50 ml media containing 1.27×10^8 suspend cells on top of the matrix of BelloCell-500.

Bring the bottle and lock up on the BelloStage console in incubator at 37℃, 5% CO_2 and start the run immediately. Avoid swirling or shaking the bottle before start compression.

Immobilization Set up operation 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 3.5 hours, switch the parameters to culture parameters. The culture control parameters are set as below:

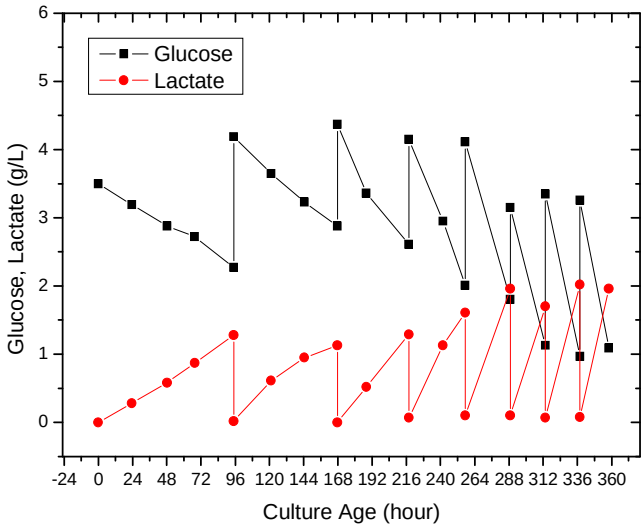
Up rate	Top holding time	Down rate	Bottom holding time
1.5 mm/s	0 sec	1.5 mm/s	1 min 30 sec

Monitor the residual glucose concentration and the color of medium in order to predict the time to change culture medium. *The setup parameters are only for reference. It does not necessary to be optimum parameters.*

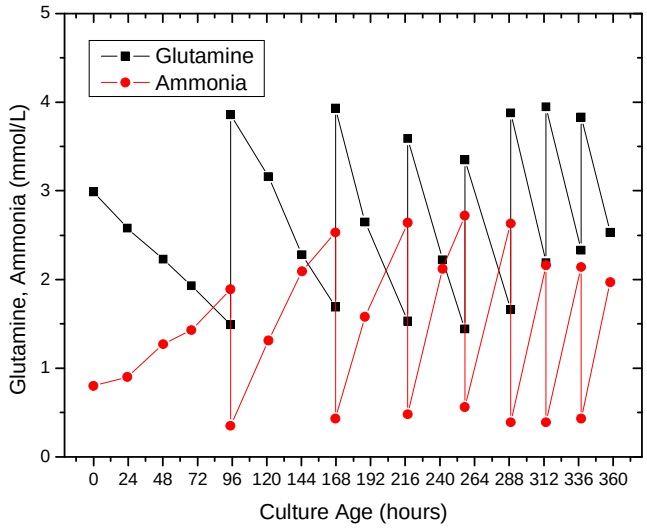
Cell Harvest The cell harvest was followed according to the protocol on the CD manual.

Results

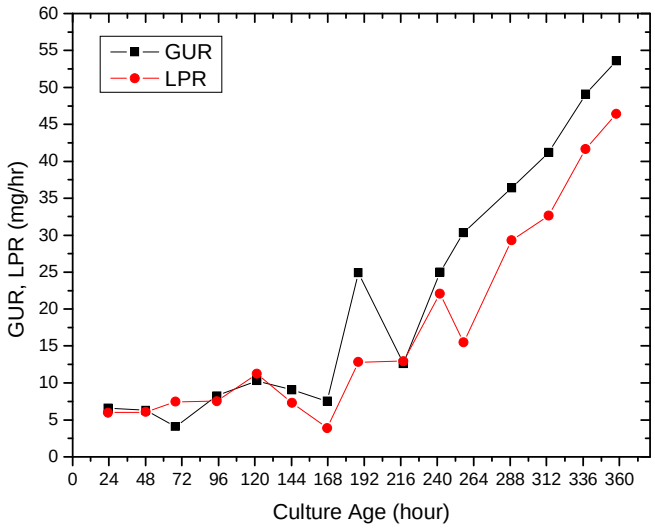
Glucose and Lactate profile



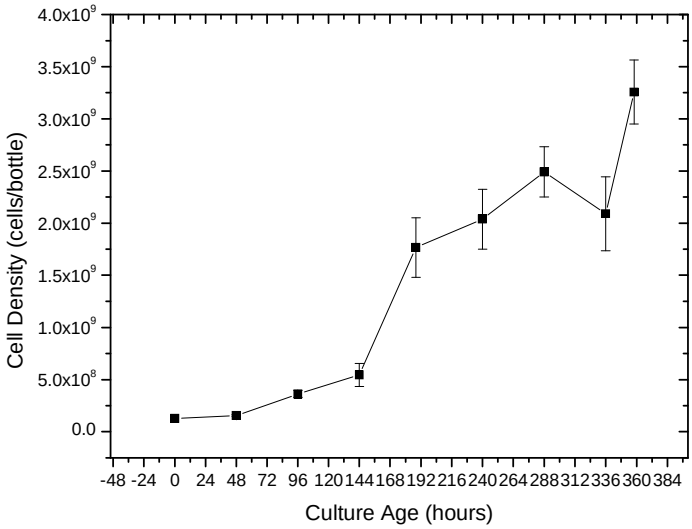
Glutamine and Ammonia profile



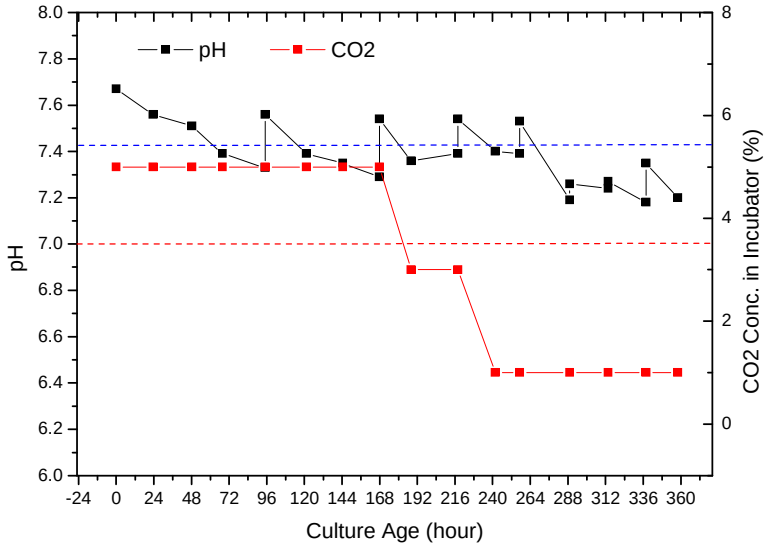
Glucose uptake rate and Lactate production rate profile



Cell grow curve by crystal violet dye nuclear count method



PH/CO2



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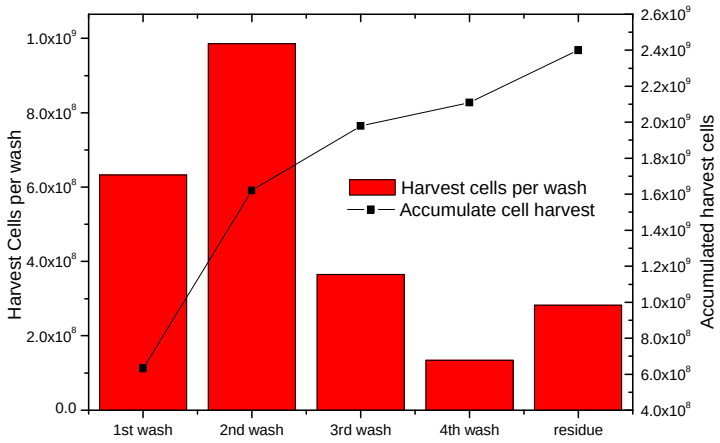
Cell Harvest



The culture of HEK 293 cells in BelloCell-500 is very smooth and the grow rate is slower than the other commonly used cell line, it require 7 days to have nearly 20 folds increase of cell population. The maximum cell density in BelloCell-500 system for HEK293 cells is around 3.5 $\times 10^9$ cells/bottle, and will require 12 days culture to reach this value. For adenovious production, we suggest to seed cells with 2×10^8 cells/bottle, and culture for 6~7 days until cell density reach above 2×10^9 cells before start infection.

Note

HEK293 cell is very sensitive to trypsin and easy to detach. Over trypsinizing the cells will cause the difficulty for cells to be immobilized in the bottle and cause a result of slow growth or even fail to growth. To avoid this, try to minimize cell dissociation process by shortening the trypsinization incubation time (within 3 mins) and terminate the enzymatic reaction by adding serum or trypsin inhibitor. CESCO also develops another bottle to enhance cell immobilization efficiency, i.e. BelloCell-500AP. If users are interested with the product, please contact CESCO Bioengineering (info@cescobio.com.tw) directly or your local distributors.

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Summary

Seed	Inoculum volume	Medium volume	Medium
1.27×10^{8} cells/bottle	50 ml/bottle	500 ml/bottle	αMEM/10%FCS
Total culture age	**Total medium consumed**	**Total medium replenish frequency**	**Final cell density (nuclear count)**
356 hrs	3500 ml	6	3.25×10^{9} cells/bottle

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

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