• BelloCell-500 Technical Report IX •

Cultivation of BHK-21 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 BHK-21 cells is illustrated. 1.12×10^8 cells/bottle was seeded and obtain a total of 5.51×10^9 cells counted by crystal violet dye nuclei count method at 188.5 hours, with a total 49 folds increase of cell population. 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	BHK-21	DMEM/10%FBS, glucose 4.5g/L, glutamine 6 mmol/L, NaHCO3 2.2 g/L	1.12×10^8 cells/bottle

Protocol **Detail protocol is in General Instruction Manual*

Inoculum preparation Prepare two T-150 flasks. Seed with 7.5×10^6 cells total. Culture at 37℃, 5% CO_2 for total 3 days. Harvest cells by standard trypsinization protocol. Prepare 1.12×10^8 suspend cells, and concentrate cells in 50 ml culture medium. **Inoculation** Pre-warm DMEM/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 the bottle. Dispense 50 ml media containing 1.12×10^8 suspend cells that has been prepared previously on top of the matrix of BelloCell-500. Bring the bottle and lock up on the BelloStage console immediately 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 secs	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:

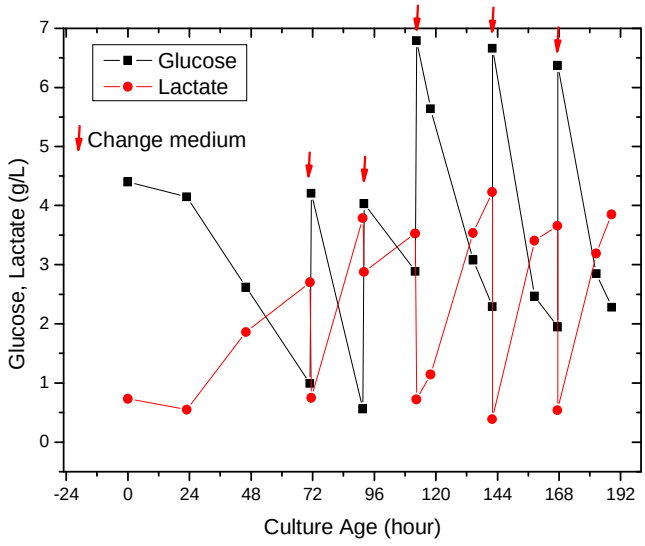
Up rate	Top holding time	Down rate	Bottom holding time
1.5 mm/s	0 sec	1.5 mm/s	1 min 30 secs

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.*

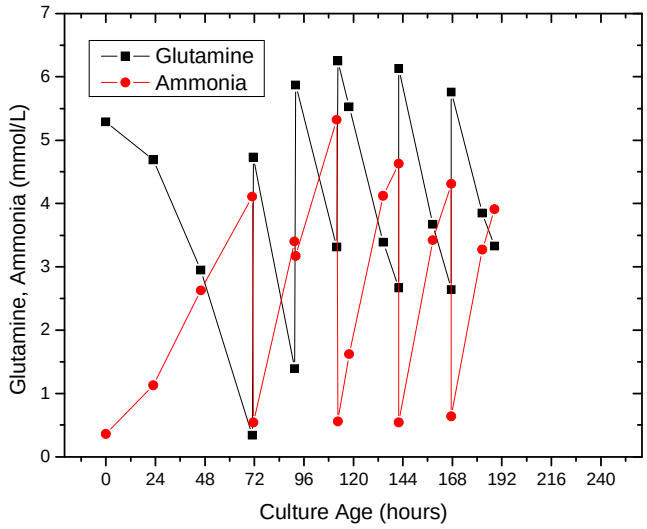
Cell Harvest The cell harvest was followed according to the protocol on CD manual

Results

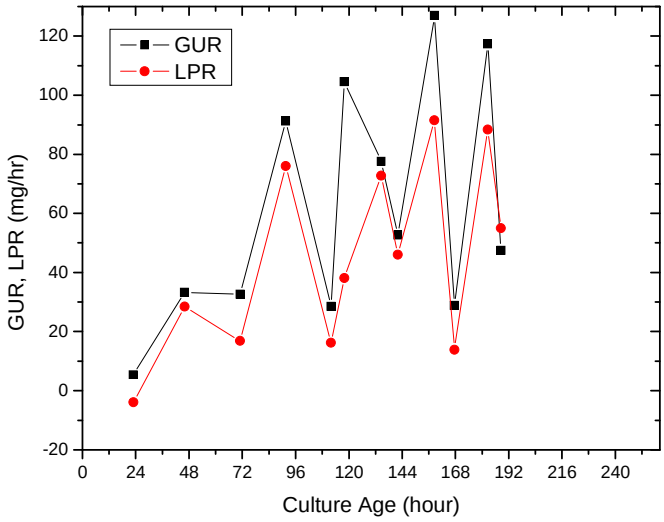
Glucose and Lactate profile



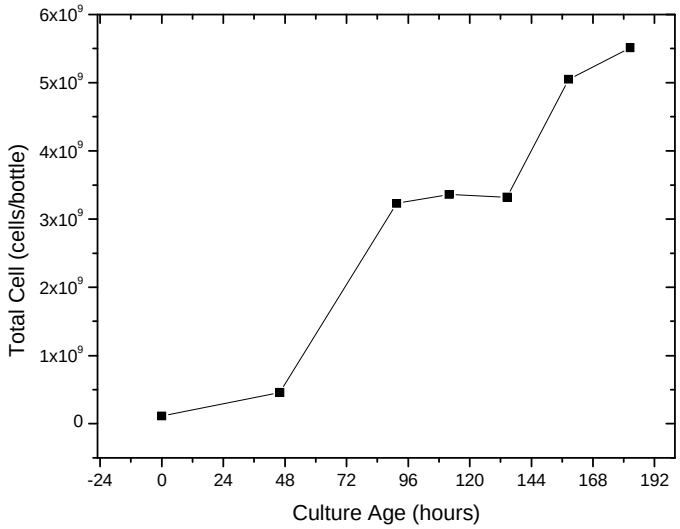
Glutamine and Ammonia profile



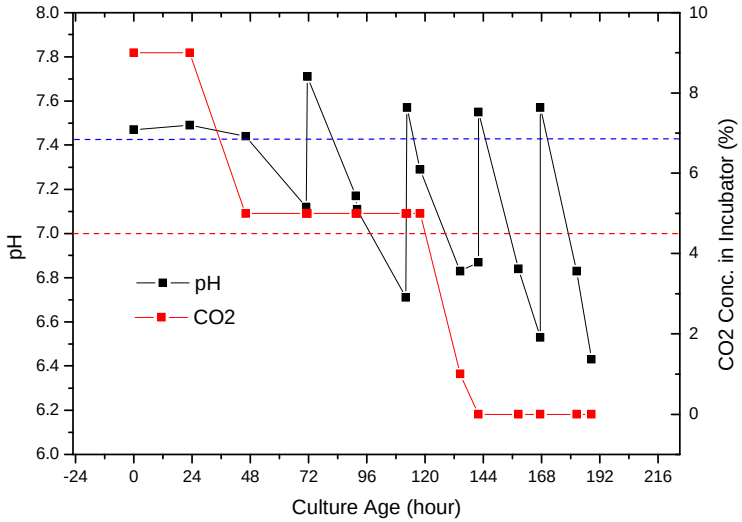
Glucose uptake rate and Lacate production rate profile



Cell grow curve by crystal violet dye nuclei count method



PH/CO2



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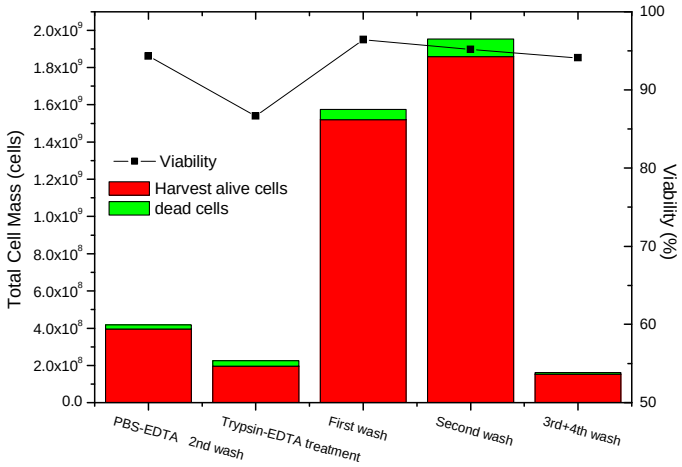
Cell Harvest



The growth of BHK-21 cells in BelloCell-500 is very fast, it requires only 8 days to have nearly 50 folds increase of cell population. The maximum cell density in BelloCell-500 system for BHK21 cells is around 5.5×10^9 cells/bottle. PH is out of control during late phase of culture. We suggest to control the initial glucose concentration to below 3 g/L in order to control the pH within range. For cell harvest, most cells (90%) could be collected before 3rd time of wash with 95% viability.

Summary

Seed	**Inoculum volume**	**Medium volume**	**Medium**
1.12×10^8 cells/bottle	50 ml/bottle	500 ml/bottle	DMEM/10%FCS
Total culture age	**Total medium consumed**	**Total medium replenish frequency**	**Final cell density (nuclei count)**
188.5 hrs	2500 ml	4	5.51×10^9 cells/bottle

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

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