CelCradle-500 Technical Report IX

Cultivation of BHK-21 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 BHK-21 cells is illustrated. 1.12 x 10^8 cells/bottle was seeded and obtained a total of 5.51 x 10^9 cells 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.

2 Material

Device	Cell Line	Medium	Seed
CelCradle-500	BHK-21	DMEM/10%FBS, Glucose 4.5g/L, Glutamine 6mmol/L, $NaHCO_3$ 2.2g/L	1.12 x 10^8 cells/bottle

3 Protocol

3.1 Inoculum Preparation

Prepare two T-150 flasks. Seed with 7.5 x 10^6 cells total. Culture at 37℃, 5% CO_2 for a total of 3 days. Harvest cells by standard trypsinization protocol. Prepare 1.12 × 10^8 suspend cells, and concentrate cells in 50 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 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 CelCradle-500. Bring the bottle and lock up on the CelCradle console immediately in incubator at 37℃, 5% CO_2 and start the run immediately. Avoid swirling or shaking the bottle before start compression.

3.3 Immobilization

Set up operation 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

3.4 Culture

After 3.5 hours, switch the parameters to culture parameters. The culture control parameters are set as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
1.5 mm/s	0 sec	1.5 mm/s	90 sec

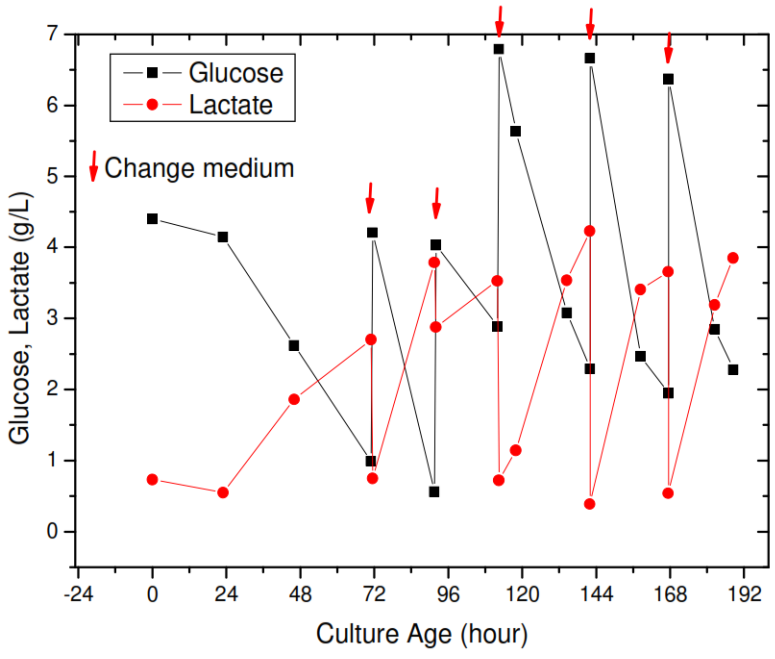
Monitor the residual glucose concentration and the color of medium in order to predict the time to change culture medium.

3.5 Harvest

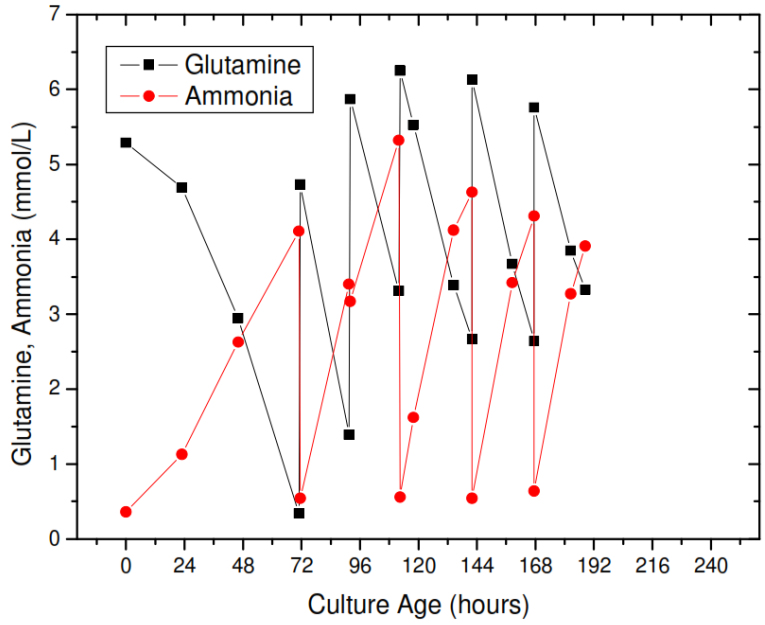
The cell harvest was followed according to the protocol on CD manual.

4 Result

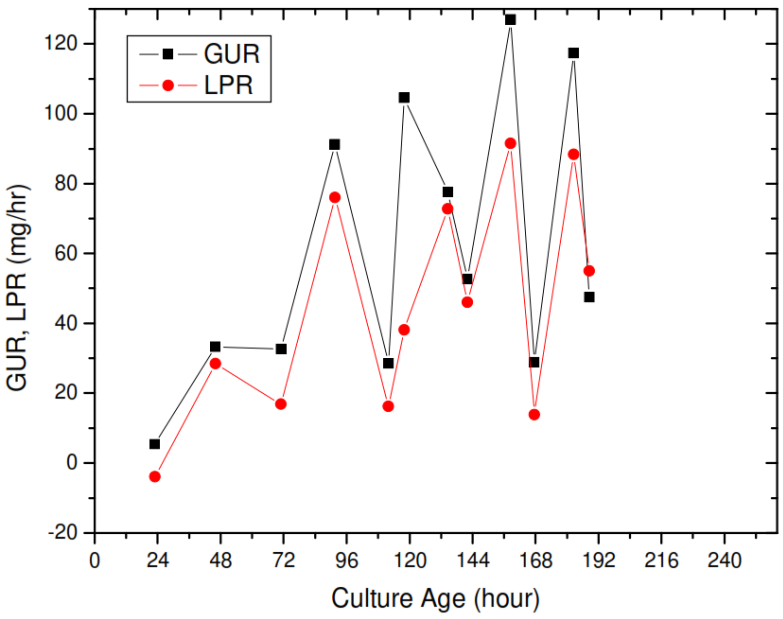
Glucose and Lactate Profile



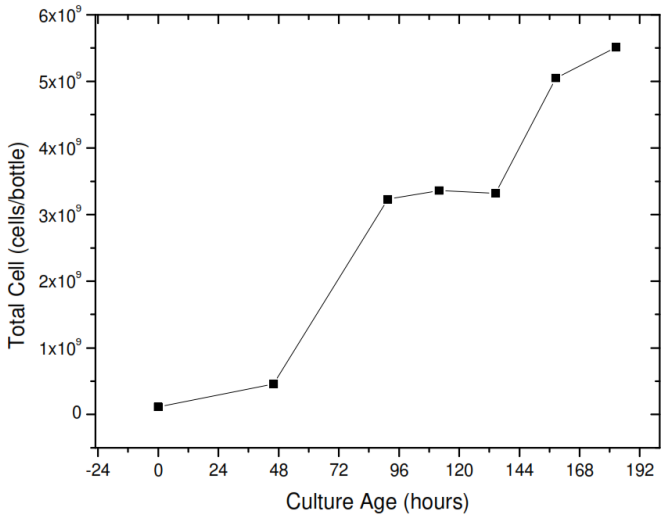
Glutamine and Ammonia Profile



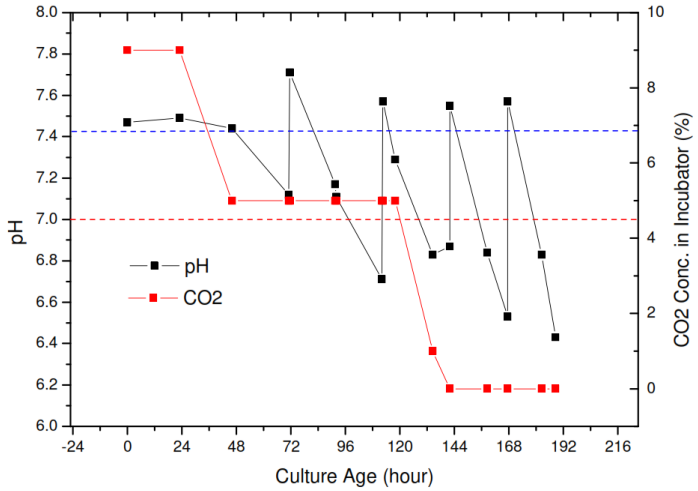
Glucose uptake rate and Lacate production rate profile



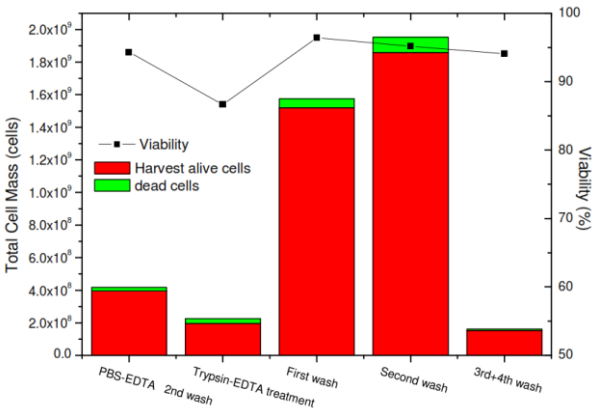
Cell grow curve by crystal violet dye nuclei count method



pH/CO_2



Cell Harvest



The growth of BHK-21 cells in CelCradle-500 is very fast, it requires only 8 days to have nearly 50 folds increase of cell population. The maximum cell density in CelCradle-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 3 time of wash with 95% viability.

5 Summary

Seed	Inoculum Volume	Medium Volume	Medium
1.12 x 10^8 cells/bottle	50 ml	500 ml	DMEM/10%FCS
Total Culture Age	**Total Medium Consumed**	**Total Medium Replenish Frequency**	**Final Cell Density (Nuclei Count)**
188.5 hours	2500 ml	4	5.51 x 10^9 cells/bottle

6 VacciXcell Technical Support

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